Redox Physiology of Elite Endurance Athletes

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A dissertation submitted to the Department of Health and Human Performance,
College of Liberal Arts and Social Sciences in partial
fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in Kinesiology

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August 2022

To Mom and Dad for cultivating my passion. To my Wife for sharing the journey. To my Son and Daughter for wherever they may carry the torch.

“Science and everyday life cannot and should not be separated.”

-Rosalind Franklin

ACKNOWLEDGMENTS

I would like to genuinely thank my co-chairs Dr. Simpson and Dr. Ledoux who have been instrumental throughout this process. Dr. Simpson honorably stuck with my cohort long after his University of Houston tenure, all the while treating us as colleagues until we earned that place. Dr. Ledoux has been my voice of reason and vital navigator for the greater half of this journey, without whom I may never have returned to solid ground. I'd also like to thank Dr. O'Connor for his prodigious guidance and Dr. Park for my introduction to ROS which has blossomed into a search for oxidative balance. And to Dr. Yellen, I would like to express my indebted appreciation for his overtly hidden words of wisdom that I didn't understand until I needed them. And an epochal thanks and deep gratitude to Dr. Pedlar and the Orreco team for their exceptional perspectives, continued collaboration, and pioneering science.

I'd like to commend my whole cohort for making this experience what it was, especially my fellow lab members, Dr. Forrest Baker, Dr. Junyoung Hong, Dr. Hawley Kunz, Dr. Jong-hae Lee, Dr. Rachel Levine, Dr. Leo Mylabathula, Dr. Justin Reed, Dr. Bridgette Rooney, and most notably Dr. Nadia Agha, who was my fundamental ally from start to finish. And to that end I would also like to thank all the people from over the years who led me down the path to the start: Dr. Alfredo Canhoto, Dr. Phillip Danielson, Dr. Jim Fogleman, Dr. Bessie M. Vaughns, Dr. Joe Vigil and Dr. McDonald to name a few.

Finally, I would like to thank my family. My mother Carol and my father Hossein were both the first in their respective families to earn a college degree, so it's no surprise the reverence for education which they have instilled. Among the expanse of their guidance and influence, my dad's patented salami approach has served me well, cutting my tasks down to manageable

bites so that I don't choke myself. And my mom's inveterate regard for the *Golden Rule* has allowed me to approach those tasks with more than just myself in mind. Even though I now have children of my own, I still strive to make you proud. Thank you.

To my wife Emma, thank you for dozing the obstacles, leading the team and making this process your own. Without all that, simply none of this would have been possible. And to my children, Rocky and Sora, you inspire me every day to be the best version of myself and I hope you find the same in your odyssey to make this world a better place.

ABSTRACT

As participation in sports and physical activity continues to thrive around the globe and athletes continue to push their limits to compete at the highest level, sport related injury and illness have become a concern. It is well known that illness and injury increase with athlete training load, however, so does performance. That leaves athletes and their support team constantly trying to find the edge of their physical and physiological training tolerance - especially elite and professional athletes. Despite great advances in science and technology, training load is still primarily managed via trial and error and subjective athlete feedback measures such as self-perception of mood, fatigue, soreness, and exertion. Because biomarkers of redox balance in the peripheral blood supply have shown early promise as a quantitative measure to assess the physiological response to training and competition, we sought to determine the effects of semi-acute physiological stressors on point-of-care (PoC) biomarkers of redox balance in a sample of elite endurance track athletes and compare those values to reported clinical values. The aims of this dissertation were divided into four hypotheses. First, despite extensive training, PoC redox biomarkers will reveal significantly lower oxidative stress in elite endurance track athletes relative to published values associated with clinical (disease) states. Second, because relative training load is typically the highest during pre-season training of endurance track athletes, (general preparation periodization phase), PoC redox biomarkers will show significantly higher oxidative stress during pre-season training relative to other training phases throughout the year. Third, periods of altitude training will be associated with significantly higher oxidative stress than sea level training. And finally, mRNA COVID-19 vaccination (SPIKEVAX™ (mRNA-1273), Moderna Co., Cambridge, MA, USA) will be associated with a significant but transient increase of oxidative stress and inflammation [i.e,

high sensitivity C-Reactive Protein (hsCRP)]. To test the first hypothesis, PoC redox biomarker values collected from 24 elite endurance track athletes over a 5-year period were analyzed by athlete and compared to published data on independent samples involving clinical conditions. To test the second hypothesis, the PoC redox biomarker values collected from 24 elite endurance track athletes over a 5-year period were analyzed by month over the course of the competitive year to evaluate alterations in redox balance relative to time-in-season. To test the third hypothesis, PoC redox samples collected for eight elite endurance track athletes throughout the course of an altitude training camp were analyzed for alterations after 5, 12, 19 and 26 days at altitude relative to athlete specific sea-level baseline values. To test the fourth hypothesis, PoC redox biomarkers as well as hsCRP samples collected for nine elite endurance track athletes prior to and throughout the course of mRNA vaccination were analyzed for alterations in redox balance. In support of our first hypothesis, it was found that mean oxidative stress levels in elite endurance track athletes were lower than reported values for clinical states, but not significantly different than healthy controls or most other characterized athletic populations. Additionally, when alterations in athlete redox balance exceeded clinically relevant levels, the response was predominantly dynamic, quickly returning to within normal ranges. In support of our second hypothesis, it was observed that oxidative stress (i.e., OSI) was elevated during pre-season training of elite endurance track athletes, particularly in December (i.e., General Preparatory training period). This alteration in redox balance was primarily driven by a decrease in anti-oxidative capacity (i.e., FORD). Contrary to our third hypothesis, no significant alteration in redox balance was detected over 26 days of training at altitude in our sample of athletes. And in support of our final hypothesis, it was observed that COVID-19 mRNA vaccination transiently increased oxidative stress (i.e., OSI) 6 days after

the first vaccine dose, with a reduction in anti-oxidative capacity (i.e., FORD) that persisted throughout the inter-dose period. Additionally, a stark but transient rise in hsCRP was detected in the first 6 days after the first COVID-19 mRNA vaccine dose in some by not all athletes. From these results, we conclude that PoC redox biomarkers are a valuable objective means to monitor the physiological condition and resilience of athletes, especially for high level programs where professional longevity depends on continued health, minute fluxuations in athlete performance are meaningful, and sport scientists are available to interpret the data from this pioneering bio-analytical method.

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Chapter 1

Introduction

Study Rationale

Bloodborne biomarkers are the staple objective screening tool for health and wellness in and out of sport . Biomarkers of oxidative stress have been developed to assess the balance between oxidation and reduction reactions (i.e., redox reactions) within the peripheral blood supply, as a measure of physiological balance (D. P. Jones, 2006). Biological redox reactions are intimately linked to cellular respiration and energy utilization and as such encompass a complex and dynamic system that is central to human physiology (Sies, 2015). The dynamic nature of redox biochemistry is what makes biomarkers of oxidative stress particularly appealing as indicator of internal physiological balance in terms of heath or disease (Allegra, 2021; Sanchez-Rodriguez & Mendoza-Nunez, 2019) or as a determinate of resilience upon challenge via physiological stressor such as a clinical treatment (Weniger et al., 2011), exercise bout (Lewis, Towey, Bruinvels, Howatson, & Pedlar, 2016) or environmental conditions such as heat (Jordan, Perry, & Cheng, 2021), cold (Martarelli, Cocchioni, Scuri, Spataro, & Pompei, 2011), high altitude (i.e., hypoxia) (Mrakic-Sposta et al., 2021; Raberin et al., 2021), pollutants (Linhartova, Gazo, & Sampels, 2016; Lodovici & Bigagli, 2011), hyperbaria (Faiss et al., 2013; Mrakic-Sposta, Vezzoli, et al., 2020; Perovic, Sobocanec, Dabelic, Balog, & Dumic, 2018; Ribon et al., 2016), and microgravity (Pavlakou, Dounousi, Roumeliotis, Eleftheriadis, & Liakopoulos, 2018). Oxidation reactions and biomarkers of oxidative stress have been

evaluated in the context of exercise physiology for almost 100 years with respect to energy metabolism (Baumberger, Jurgensen, & Bardwell, 1933; Harden & Henley, 1922; Harrison, 1932; Sheldon, Johnston, & Newburgh, 1937; Wierzuchowski, 1937) and for over 30 years in terms of (Campos, Gomes, & Ferreira, 2013) exercise (Terrados, 1992; Vasankari, Kujala, Rusko, Sarna, & Ahotupa, 1997) and environmental stress (Gohil, Viguie, Stanley, Brooks, & Packer, 1988; Smith, Kolbuch-Braddon, Gillam, Telford, & Weidemann, 1995; Viguie et al., 1993). Redox biochemistry is an intrinsic component of energy metabolism (Campos et al., 2013) as is the cogeneration of reactive oxygen species (ROS) (Zhao, Jiang, Zhang, & Yu, 2019) therefore the increase in respiration and cellular energy utilization associated with exercise make monitoring of oxidative stress particularly suited for exercise studies and athletes. Oxidative signaling (i.e., oxidative eustress) is believed to play an essential role in the physiological adaptation to exercise, with optimal exercise induced oxidation associated with increased mitochondrial biogenesis, endogenous antioxidant production, angiogenesis, vasodilation, insulin signaling, growth factor signaling, and increased antioxidative capacity (Hadzovic-Dzuvo et al., 2014; Jordan et al., 2021). Yet, excessive, or continued elevation of oxidative status (i.e., oxidative distress), is associated with reduced antioxidative capacity, irreversible damage to cellular components, overtraining, fibrinogenesis, cell death and disease (Dionisio, Amaral, & Rodrigues, 2021; Jordan et al., 2021; Margonis et al., 2007; Tada & Suzuki, 2016; Zahra et al., 2021), therefore, monitoring of this oxidative stress balance has great potential as a tool to optimize health and performance. Extensive work has been performed to uncover the value of oxidative stress biomarkers in sport (Banfi et al., 2006; Deminice et al., 2010; Margaritelis et al., 2018), and recent research has shown biomarkers oxidative stress to have great promise as a quantitative measure of training stress in athletes

(Becatti et al., 2017; Bellafiore et al., 2019; Zainudin, Caszo, Knight, & Gnanou, 2019). Unfortunately, to date, the delay in redox biomarker assay results, the need for venipuncture and a lack of characterization of optimal ranges across differential populations and sport disciplines have hindered application as an active management tool (Pedlar, Newell, & Lewis, 2019). However, due to recent improvements in test speed, invasiveness and portability, the potential to use bloodborne biomarkers of oxidative stress as a near real-time tool to both monitor and actively manage the physiological response to training has emerged (McKay et al., 2021).

Problem Statement

As participation in sports and physical activity continues to thrive around the globe (Hulteen et al., 2017) and athletes continue to push their limits in an effort to compete at the highest level (Bolling, Delfino Barboza, van Mechelen, & Pasman, 2020), sport related injury and illness have become a health concern worldwide (Rubio, Quartiroli, Podlog, & Olmedilla, 2020). During the 19 days of competition at the 2016 Rio Olympic Games alone, 651 illnesses and 1101 injuries were reported among athletes (Soligard et al., 2017), with similar numbers (758 illnesses and 1361 injuries) reported at the 2012 London Olympics (Engebretsen et al., 2013). And this trend is not limited to the Olympic games as research has shown illness and injury prevalence across major supporting events and sports (Bullock et al., 2021; Lhee et al., 2021; Schwellnus et al., 2016). It is well known in the elite sport community that illness and injury increase with increasing training load (C. M. Jones, Griffiths, & Mellalieu, 2017; Ristolainen, Kettunen, Waller, Heinonen, & Kujala, 2014; Sugimoto, Jackson, Howell, Meehan, & Stracciolini, 2019), the problem is, so does performance (Tonnessen et al., 2014;

West et al., 2020). And despite great advances in technology and science, training load is still primarily managed via trial and error and subjective athlete feedback measures such as self-perception of mood, fatigue, soreness, and exertion (Bolling et al., 2020; Saw, Main, & Gastin, 2016). In the search for better objective measures, analysis of oxidative stress has emerged as a promising quantitative biomarker to assess the biological response to training and racing, which could allow coaches and medical staff to better optimize training load to maximize performance while minimizing injury and illness (Lewis et al., 2020). Markers of blood plasma oxidative stress has been shown to be responsive to acute exercise stress (Lewis, Towey, et al., 2016; Mrakic-Sposta, Gussoni, et al., 2020; Tanskanen, Atalay, & Uusitalo, 2010) as well as semi-acute and chronic training stress (Becatti et al., 2017; Le Moal et al., 2016; Lewis, Howatson, Morton, Hill, & Pedlar, 2015; Lewis, Towey, et al., 2016; Tanskanen et al., 2010; Tong et al., 2016). Bloodborne biomarkers of oxidative stress have also been shown to have both a positive association with athletic performance (Deminice et al., 2010; Schippinger et al., 2009; Vezzoli et al., 2016) and a negative association with injury and illness (Lewis et al., 2020), but due to the constraints of analytic methods (i.e., remote labs, lengthy processing time and invasive venous blood sampling), application has been limited to retrospective analysis. However, due to recent advances in technology and application, oxidative balance can now be assessed near real-time, at the location of the athlete (i.e., at the point-of-care), with only the need for a capillary blood sample, opening the possibility to assess an athlete's physiological condition before and even during training to more tightly regulate training application (McKay et al., 2021). However, work to determine if PoC oxidative stress biomarkers can be reliably applied as objective measures for both health and exercise is still emerging. Foundational work using these PoC biomarkers has shown them to be reliable analytical measures that correlate

well with historical lab-based measures, as well as reliably respond to acute exercise (Lewis, Newell, Burden, Howatson, & Pedlar, 2016; Lewis, Towey, et al., 2016; Quinn, Cox, Roberts, Briskey, & Minahan, 2020). And recent work has shown these PoC biomarkers to be directly associated with injury and illness in Olympic rowers (Lewis et al., 2020) as well as significantly correlate with standard subjective measures of fatigue and soreness in collegiate football players, (McKay et al., 2021). However, to date there is little known about the effectiveness of these measures in elite endurance track athletes, the clinical relevance of values seen in athletes or the effect of physiological stressors such as time-in-season, vaccination, or altitude exposure. Therefore, the overarching aim of this research is to determine how different conditions affect PoC biomarkers of oxidative stress in elite endurance track athletes and compare those values with disease populations and sedentary controls. The overarching hypothesis is that elite endurance track athletes will display increased oxidative stress levels in response to semi-acute physiological stressors (e.g., training phase, altitude exposure, vaccination) yet will exhibit significantly lower mean values than disease populations, but not sedentary controls. It is anticipated that this work will provide additional support for the use of PoC oxidative stress as a suitable objective biomarker to monitor the physiological condition of athletes to aid in training load management to help reduce the risk of underperformance, illness, and injury.

Research Questions & Rationale

Research question 1: Do elite endurance track athletes exhibit lower levels of PoC bloodborne biomarkers of oxidative stress relative to disease populations and sedentary controls?

Rationale: Extensive aerobic training is known to increase levels of oxidative stress, however the severity of the exercise induced oxidative stress relative to levels associated with disease is not well understood.

Research question 2: Are there seasonal alterations in the redox balance of elite endurance track athletes over the course of the competitive year?

Rationale: Elite athletes have been shown to experience seasonal alterations in redox balance with elevated levels of oxidative stress linked to lower performance, illness and injury, yet the annual alterations in redox balance of elite endurance track athletes have not been well characterized.

Research question 3: Is altitude training associated with increased levels of PoC bloodborne biomarkers of oxidative stress relative to training at sea level in elite endurance track athletes?

Rationale: Altitude training is a key component of elite endurance athlete training program because of the potential to increase oxygen carrying capacity but has also been shown to increase oxidative stress as well as rates of illness. However, PoC biomarkers of oxidative stress associated with altitude training of elite endurance track athletes have never been reported.

Exploratory research question: Is mRNA COVID-19 vaccination associated with an increase in PoC biomarkers of oxidative stress in elite endurance track athletes?

Rationale: Both SARS-CoV2 infection and mRNA COVID-19 vaccination have been associated with increased levels of oxidative stress, as well as the oxidative stress related

conditions such as myocarditis and pericarditis, however neither the effect of mRNA vaccination on athletes, nor PoC biomarkers of oxidative stress have been reported.

Literature Review

Redox Biology

Redox biochemistry is fundamental to human physiology, as electron exchange, gradients and flow are at the heart of biological life (Herrmann & Dick, 2012). Redox biology involves the study of biological reactions where there is a transfer of one or more electrons between two biomolecules - the electron donor biomolecule is 'oxidized' with the loss of an electron and the recipient biomolecule is 'reduced' (Herrmann & Dick, 2012). This exchange of electrons typically happens in biochemical reactions with the transfer of hydrogen atoms (and their electron) from a more mildly electronegative atom such as carbon to the more highly electronegative atoms of oxygen or nitrogen. This hydrogen electron exchange is a dynamic and ever-changing process and therefore both the oxidation and reduction aspects are usually summarized simply as 'redox' (Franco & Vargas, 2018).

Oxidative Species. As a byproduct of normal biological functions such as aerobic metabolism, oxidative and highly reactive oxygen or nitrogen species (ROS or RNS) are formed. The main endogenous types of ROS include superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH-), and the main endogenous types of RNS include nitric oxide `(NO-) and peroxynitrite (OONO-), and together they can be referred to as RONS or collectively as ROS since all RNS also contain one or more oxygen molecules. Collectively, all of these ROS have inherent chemical properties that confer high reactivity with various biological targets (Schieber & Chandel, 2014). This reactivity is further increased and can be particularly delirious if the newly formed biomolecule has one or more unpaired valence electrons, as is the case with hydroxyl radicals (OH-), superoxide anions (O_2-), nitric oxide

(NO-) and peroxynitrite (OONO-) (Borden, Hoffmann, Stuyver, & Chen, 2017). The inherent reactivity of these reactive [oxidative] species (RS) is vital to a multitude of biological process such as proliferation of endothelial and erythroid progenitor cells via HIF-1α stabilization (Diebold & Chandel, 2016), cell signaling mechanisms (Brand, 2016) and tissue homeostasis (Ferreira, Ni, Rosenkrans, & Cai, 2018), yet excessive amounts can cause a multitude of detrimental effects including lipid peroxidation, DNA damage, cellular functional abnormalities due to protein oxidation, mitochondrial damage, myelin catabolism, disruption of ATP generation and even cell death (Baraibar, Liu, Ahmed, & Friguet, 2012; Klosinski et al., 2015; Orrenius, Gogvadze, & Zhivotovsky, 2007). As essential by-products of aerobic energy metabolism via the electron transport chain (Di Meo & Venditti, 2001), ROS are intrinsically tied to exercise (Schieber & Chandel, 2014; Zhao et al., 2019). Historically ROS were regarded as deleterious to cells (Mason, Morrison, McConell, & Wadley, 2016), but research over the last few decades had continued to demonstrate their vital importance as redox signaling constituents, which has given rise to a new era of redox biology where balance is essential for optimal cellular function (Zimmerman & Case, 2019), particularly the balance between ROS generation and the reductive potential of the cellular microenvironment (Gomez-Cabrera, Vina, & Olaso-Gonzalez, 2020). With oxygen as the final electron acceptor of the electron transport chain (ETC) within the mitochondria, electron flow to the final ETC complex (complex IV) results primarily in the production of H_2O, however with the increased energy demand of endurance exercise, ROS in the form of superoxide (O_2-) is produced (primarily in complex I and III) at a rate of up to 3% (Vargas-Mendoza et al., 2021). The anion superoxide is formed by the one-electron reduction of molecular O_2, and is the main ROS formed in cells. Superoxide is unstable and impermeable to the cell membrane, however it can

undergo dismutation to form hydrogen peroxide which is more stable and can diffuse across the cell membrane and therefore is the ROS form believed to be responsible for the majority of cell signaling (Vargas-Mendoza et al., 2021). H_2O_2 together with O_2- are transformed into hydroxyl radicals (OH-), which are also highly reactive and impermeable to the cell membrane. The presence of the unpaired electron in the valence shell of superoxide and hydroxyl radicals, as indicated by the inherent negative charge, classifies these molecules as "free radicals" and is what confers their high reactivity; these molecules typically only exist transiently as the unpaired valence electron encourages rapid stabilization via further covalent bonding (Borden et al., 2017). On the other hand, the hydrogen peroxide formed is more stable, can be accurately quantified, is diffusible and can be secreted and imported into cells to initiate oxidative signaling mechanisms such as oxidative inhibition of tyrosine phosphatases, which in turn increases tyrosine phosphorylation on target proteins (Boveris, Oshino, & Chance, 1972; Knaus, 2020). These structural and functional properties inherent to hydrogen peroxide make it the primary signaling molecule in redox processes and thus a preferred biomarker target for monitoring of oxidative balance (Knaus, 2020; Sies, 2017). However, the increase in pro-oxidative process via ROS generation only accounts for half of the redox balance, as the reductive or anti-oxidative protentional of the microenvironment playing an equally important role (Radak et al., 2017).

Anti-Oxidative Processes. Anti-oxidative aspects of the redox balance play an elemental role in redox biology as the reductive or anti-oxidative potential of the cellular microenvironment must be aligned with pro-oxidative processes for optimal health (Hu et al., 2019) and performance (Leon-Lopez et al., 2018; Sousa et al., 2019). It is also particularly interesting from an applied science perspective as ROS generation seems to be largely

biologically intrinsic and stimulus modulated (Wada, Takeda, & Kuwahata, 2017), whereas some anti-oxidative aspects can be pro-actively managed via nutrition (Dennis, Go, & Jones, 2019). This is the basis of the mechanistic segregation of the anti-oxidative elements of redox biology, which are typically segregated in to inherent (i.e., endogenous) and externally influenced (i.e., exogenous) aspects (Di Pierro et al., 2020). These aspects can be further broken down to endogenous enzyme systems and anti-oxidative small molecule production and exogenous nutritional antioxidants and co-factors (Di Pierro et al., 2020; Mason, Trewin, Parker, & Wadley, 2020). Endogenous enzyme systems are believed to play the most prominent role in anti-oxidative capacity and include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), cytochrome 5b reductase, thioredoxin and peroxiredoxin 2 (Prx2) (Di Pierro et al., 2020; Scioli et al., 2020). Endogenous antioxidant small molecules include uric acid, coenzyme Q, and bilirubin and exogenous nutritional antioxidants is an ever-growing list that includes vitamins A, C, E, flavonoids and carotenes among others (Scioli et al., 2020). Exogenous nutritional co-factors include trace minerals necessary for the endogenous enzyme systems to function, most notably including zinc and selenium, as well as lower requirements of magnesium and copper (Alghobashy et al., 2018; Faghfouri et al., 2021; Fernandez-Lazaro et al., 2020).

Altogether, human redox biology is fundamentally the study of the ever-shifting balance between ROS production and scavenging (Khomich, Kochetkov, Bartosch, & Ivanov, 2018) quantified in terms of indirect biomarkers of acute ROS generation (e.g., peripheral blood hydrogen peroxide levels) and anti-oxidative potential (e.g., Total Anti-Oxidative Capacity (TAC) (Sies, 2020), in addition to markers of oxidative damage to endogenous

nucleic acids, lipids and proteins (Assi, Dufresne, & Rebillard, 2020; Isaguliants, Bartosch, & Ivanov, 2020).

Redox Exercise Physiology

The increased metabolic demand associated with both acute and chronic exercise elicits a wide range of overlapping stress-adaptive responses which are largely modulated via redox signaling events (Henriquez-Olguin, Meneses-Valdes, & Jensen, 2020). Exercise induced RS are essential upstream signals for the activation of transcription factors and the induction of gene expression associated with exercise (Gomez-Cabrera, Domenech, & Vina, 2008). These signaling events are essential to the primary physiological adaptations to training, including mitochondrial biogenesis, vascular angiogenesis, skeletal muscular hypertrophy, cytoprotection and redox homeostasis (Gomez-Cabrera et al., 2020; Margaritelis, Paschalis, Theodorou, Kyparos, & Nikolaidis, 2020b). And although underlying mechanisms are not yet completely understood, evidence is emerging that the effectiveness of this adaptive response to exercise is dependent on the homeostatic balance of the intracellular redox environment (Cobley, Sakellariou, Husi, & McDonagh, 2019). Redox physiology is sophisticated with thermodynamics, enzyme kinetics, anti-oxidant specificity and compartmentalization at play (Henriquez-Olguin et al., 2020; Margaritelis, Paschalis, Theodorou, Kyparos, & Nikolaidis, 2020a; Nikolaidis, Margaritelis, & Matsakas, 2020), yet the fundamental prevailing theory is that the optimal redox microenvironment in terms of both physiological adaptation and disease prevention, allows for effective adaptive signaling via temporary increases in oxidative state upon stimulation via an acute stressor such as exercise, followed by a rapid return to a nominal baseline (eustress) oxidative state upon removal of the stressor (Sies, Berndt, & Jones, 2017).

In these terms, physiological conditions that result in muted redox signaling will accordingly prevent the optimal response to the stressor, and conversely, extended elevation of the oxidative state increases the destructive potential of RS and can lead to poor health and/or a disease state (Buresh & Berg, 2015; Scioli et al., 2020; Thannickal, 2020). Therefore, a reliable and easily implementable quantitative biomarker of redox balance, both in terms of oxidative state as well as reductive potential, would provide great value in optimizing the intracellular environment and maximizing health and the adaptive response to exercise (Theofilidis, Bogdanis, Koutedakis, & Karatzaferi, 2018).

Evolution of Redox Biological Science & Hormesis

Redox biology is intrinsically tied to human biology, comingling their evolution and making origins of redox biology difficult to establish (Flohe, 2020), but in regards to exercise, early investigation of redox biochemistry was largely centered around lactate metabolism as the generation of lactate during anaerobic glycolysis involves the reduction of pyruvate to lactate via the oxidation of NADH to NAD+ (the hydrogen donor), which is catalyzed by the enzyme lactate dehydrogenase (Baumberger et al., 1933; Brodan & Kuhn, 1968; Gudbjarnason & Bing, 1962; Labeyrie, Naslin, Curdel, & Wurmser, 1960). Over time, development of analytical techniques continued to evolve and quantification of redox markers became increasingly more viable (Sies, 2020), yet largely required invasive techniques such as muscle biopsies (Henriksson, Katz, & Sahlin, 1986; Sahlin, Katz, & Henriksson, 1987) or labeled radioisotopes (Mazzeo, Brooks, Schoeller, & Budinger, 1986) until more advanced enzyme kinetics assays to evaluate bloodborne biomarkers began to emerge (Gohil et al., 1988). These new techniques allowed for the identification of reactive species and the collection of more

redox data with the research at the time centered around the prevailing belief that oxidation and RS such as 'free radicals' were considered detrimental (Sastre et al., 1992; Takenaka, Miki, Yasuda, & Mino, 1991; Thomas, McLean, Parker, & Ohlweiler, 1992). This gave rise to the boom in anti-oxidant food and supplement research and the potential benefits of using them to reduce 'oxidative stress' for better health and performance (Jakeman & Maxwell, 1993; Kanter, 1994; Maxwell, Jakeman, Thomason, Leguen, & Thorpe, 1993; Sastre et al., 1992; Viguie et al., 1993). Although, soon experimental evidence began to surface that there were positive and essential aspects to these RS which led to the theory of oxidative stress hormesis proposed by Radak, et al. in 2005, which postulated that there is an optimal balance between oxidant generation and removal (Radak, Chung, & Goto, 2005; Radak, Chung, Koltai, Taylor, & Goto, 2008), beginning the modern era of research on redox and exercise (Zimmerman & Case, 2019).

The idea of biological hormesis was not a new one as it was first proposed by Southam and Ehrlich in 1943 in relation to the stress response of plants (Southam & Erlich, 1943) and has since been expanded to numerous fields, most notably pharmacology/toxicology (E. J. Calabrese, 2014), but Radak et al. was the first to apply it to redox biology. In short, hormesis has been summarized as biological adaptive process where low levels of a ligand is stimulatory, yet higher concentrations of that same ligand is inhibitory (E. J. Calabrese & Baldwin, 2002; Carelli & Iavicoli, 2002; Kendig, Le, & Belcher, 2010; Kitchin, 2002), which describes the mechanistic activity of both the oxidants and anti-oxidants in redox biology (Leak et al., 2018; Radak et al., 2017). Not surprisingly, the dose-response relationship of hormesis also accurately describes the initiative dose-response relationship of exercise stress and adaptation (Radak et al., 2008). Soon after Radak et al. first proposed the extension of hormesis to redox

biology, investigations flourished around the integral nature of oxidative signaling in physiological adaptation to exercise (Campos et al., 2013; Ferraro, Giammarioli, Chiandotto, Spoletini, & Rosano, 2014; Ji, 2008; Kang, O'Moore, Dickman, & Ji, 2009; Kruger et al., 2009; Roy, Khanna, & Sen, 2008; Scheele, Nielsen, & Pedersen, 2009), and the potential detrimental effects of exogenous high-dose antioxidants muting of the adaptive oxidative signaling (Niki, 2012; Nikolaidis, Kerksick, Lamprecht, & McAnulty, 2012; Roberts, Beattie, Close, & Morton, 2011). Much discussion remains in regards to the optimal redox balance as scientific understanding of biochemical pathways and the effect of exercise matures (Louzada et al., 2020) and characterization of redox biological variation within and between individuals (Lewis, Newell, et al., 2016) as well as between sexes (Di Florio, Sin, Coronado, Atwal, & Fairweather, 2020) and sport disciplines (Sohail et al., 2020) continues. Notwithstanding, today there is consensus that redox signaling is integral to adaptive mechanisms and that concentration and temporal balance is paramount for both optimal health and performance (Antonioni, Fantini, Dimauro, & Caporossi, 2019; Ismaeel, Holmes, Papoutsi, Panton, & Koutakis, 2019; Koivisto et al., 2019; Leak et al., 2018; Mason et al., 2016; Mason et al., 2020; Pastor & Tur, 2019; Radak et al., 2017; Reid, 2016; Rothschild & Bishop, 2020).

Exercise Effect on Redox State

Human redox research has been applied to a wide range of sports to better understand the physiology of the adaptive process to exercise (Nikolaidis, Kyparos, et al., 2012) and has revealed a complex process as moderate aerobic endurance exercise bouts often reduce markers of oxidative stress, whereas more significant endurance exercise bouts have been shown to increase oxidative stress, both in terms of oxidative balance (ROS) as well as

oxidative damage (lipid, protein or nucleotide oxidative modifications) (Radak, Zhao, Koltai, Ohno, & Atalay, 2013; Thirupathi et al., 2020). In addition, the strength of the exercise stimulus appears to be relative, as athlete age, sex, BMI, training experience, nutritional status and chronic training load affect baseline redox values as well as the response to the acute exercise stimulus (Antonioni et al., 2019; Bloomer & Fisher-Wellman, 2008; Estrela et al., 2017; He et al., 2016; Leite-Almeida et al., 2020; Lewis, Towey, et al., 2016; Seifi-Skishahr, Damirchi, Farjaminezhad, & Babaei, 2016). Although complex and seemingly contradictory, this bi-phasic pattern follows the hormetic inverted J-curve proposed by Radak et al. where low to moderate-dose stress is adaptive, yet becomes maladaptive at higher "doses" (V. Calabrese et al., 2016), both in terms of relative high acute dose, such as a half-marathon or longer event (Briviba et al., 2005; Mastaloudis, Leonard, & Traber, 2001), as well as high chronic dose, such as the high volume training of elite athletes (Lewis et al., 2015) or overtraining (Lewis et al., 2018; Margonis et al., 2007; Tanskanen et al., 2010).

In an early study of acute oxidative stress in Ultra Runners, Mastaloudis et al. detected an concomitant increase of lipid peroxidation (F_2-Isoprostaines) and disappearance of vitamin E (α-tocopherols) in blood plasma following a 50km Ultramarathon (Mastaloudis et al., 2001). Similarly, de Lucas et al. observed increased oxidative stress levels in ultra-endurance athletes after a 90km multi-sport event (trail running, mountain biking and kayaking) with significant increases in both lipid peroxidation (erythrocyte thiobarbituric acid-reactive substances (TBARS)) and plasma protein carbonyl (PC) content observed 15 minutes after finishing the event (de Lucas et al., 2014). Correspondingly, Hattori et al. detected not only a rise in oxidative stress in ultra-runners during a two-day Ultramarathon, but there was a direct inverse relationship between oxidative stress and performance with lower baseline serum ROS

concentrations associated with faster first day completion times (Hattori et al., 2009). In addition, they observed that ROS concentrations increased during the race (after the first day of racing) and returned to baseline post-race, postulating the involvement of some sort post-race "anti-oxidant defense system". Furthermore, due to their large sample size (70 male runners), they were able to identify significant between-subjects variation in baseline ROS levels and found that age and BMI were associated with increased baseline ROS levels (Hattori et al., 2009). With baseline oxidative stress levels tied to ultra-marathon performance, Samaras et al. tested a nutritional intervention and found that both the use of a carbohydrate-protein bar and concentrated tomato juice supplement over a two month period, significantly decreased baseline serum lipid peroxidation levels (thiobarbituric acid-reactive substances (TBARS)) as well as PC content (Samaras et al., 2014). Furthermore, this research showed that the protein bar intervention had the added effect of increasing a marker of anti-oxidant defense (plasma reduced Glutathione (GSH) levels) that was not observed with the tomato juice supplement, and speculated this to be due to the sulfur-containing cystine content of the whey protein (Samaras et al., 2014). Mrakic-Sposta et al. tested a group of extreme ultra-runners before, during and after a 330km trail run and observed significant increases in oxidative stress (ROS & nucleic acid oxidative damage biomarkers 8-hydroxy-2-deoxy Guanosine (8-OH-dG) and 8-isoprostane (8-isoPGF2α)) and reduction of blood TAC from pre to post race (Mrakic-Sposta et al., 2015). This research was also significant because they used a new, "micro-invasive" analytical technique to measure oxidative stress (i.e., X-band EPR, E-Scan-Bruker BioSpin, GmbH, MA USA) and EDEL potentiostat electrochemical analysis to measure blood antioxidant capacity (Edel Therapeutics, Switzerland) that allowed for rapid lab-based analysis using only capillary blood and urine as opposed to the requisite venipuncture (Mrakic-Sposta

et al., 2015). In subsequent study using X-band EPR, Vezzoli et al. compared the redox characteristics of ultra-runners that completed a 50km race versus those who completed a 100km race and found significant post-race increases in markers of oxidative stress [i.e., ROS, TAC (100km only), PC, TBARS, 8-OH-dG and 8-isoPGF2α], as well as described for the first time that the increases in oxidative stress were linear and directly related to exercise duration (Vezzoli et al., 2016). This work also showed a significant relationship between baseline oxidative stress and performance as lower baseline ROS levels, as well as higher baseline antioxidant status (i.e., TAC) were associated with increased performance in the 100km race group (Vezzoli et al., 2016).

The oxidative stress associated with ultra-endurance exercise has been well documented and shown to persist for up to a month or more post-race (Turner, Bennett, Bosch, Griffiths, & Aldred, 2014), however ultra-endurance exercise durations are not required to observe significant perturbations in oxidative stress. Briviba et al. showed increased oxidative DNA damage, as well as reduced plasma anti-oxidative capacity [i.e., ferric reducing ability of plasma (FRAP)] after both half marathon (21.1km) and full marathon (42.2km) events (Briviba et al., 2005). Duca et al. observed increases in plasma lipid peroxidation [i.e., malondialdehyde (MDA)] after a half marathon (21.1km) distance event, which they posited may have been the result of oxidative damage from increases in free iron [i.e., increased non-transferrin bound iron (NTBI)] due to the hemolysis associated with running (Duca et al., 2006). Unt et al. evaluated the oxidative stress of a non-running (i.e., ski) marathon and did not observe a significant increase in plasma oxidation post ski marathon despite the fact that the ski marathon was significantly longer on average than the running half-marathon tested by Duca et al. (i.e., 179 vs 79 minutes). This finding lends support to Duca's hypothesis that

running may be a more potent oxidative stressor due to the increased hemolysis, yet further study is needed due to differences in subjects and analytical tests. Additionally, Unt et al. observed differential changes in redox status between whole blood and red blood cell (RBC) GSH (i.e., positive changes in whole blood GSH, yet negative changes in RBC GSH values), leading to the conclusion that both should be attained for a more complete understanding of exercise induced oxidative changes (Unt, Kairane, Vaher, & Zilmer, 2008). Hessel et al. proposed oxygen radical generation of neutrophils as another possible explanation for the oxidative stress associated with running, which was indicated via increased lipid peroxidation and oxidized glutathione (GSSG), as well as decreased superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity post marathon run (Hessel, Haberland, Muller, Lerche, & Schimke, 2000). In an effort to elucidate exercise induced oxidative mechanisms, Gomez-Cabrera et al. showed that the increase in lipid peroxidation post marathon (increased plasma MDA and ROS driven activation of nuclear protein NF-κB in lymphocytes) could be arrested with the use of the xanthine oxidase (XO) inhibitor Allopurinol, which blocked XO-derived ROS formation and possibly the subsequent upregulation of protective anti-oxidant enzymes (Gomez-Cabrera et al., 2006). Thus, this research supports the role of exercise induced ROS production as an integral component in cell adaptation and that anti-oxidant administration may prevent positive adaptations induced by exercise (Gomez-Cabrera et al., 2006). Withee et al. also found increased oxidative stress associated with half-marathon running with significant increases of 8-OHdG and MDA at 15 minutes-post and 8-OHdG at 90 minutes-post half-marathon (Withee et al., 2017). They also found that the use of the sulfur based nutritional supplement Methylsulfonylmethane (MSM), taken for 21 day prior to the race, had no effect on the exercise induced oxidative changes (Withee et al., 2017). Li et al.

used well-trained male marathon runners in a randomized cross-over design experiment to evaluate the impact of normoxia (21.0% FiO_2) versus hypoxia (14.4% FiO_2) on redox status after a 92 minute intermittent exercise protocol (i.e., alternating 2 minute intervals of 90% and 50% VO_2max) and found that the hypoxia reduced anti-oxidative capacity (i.e., GSH and TAC) post exercise (Li et al., 2016). They did not see the expected elevation in lipid peroxidation (i.e., MDA) post exercise in either normoxia or hypoxia and posit that the exercise bout chosen may not have been significantly harder than the routine training of their well-trained participants and therefore not enough stimulus to observe oxidative changes (Li et al., 2016). Larsen et al. investigated the time-course changes of redox balance in recreational runners following a marathon and found an increase in nucleic acid oxidative damage [i.e., 8-oxo-7,8-dihydroguanosine (8-oxoGuo)] post marathon, but interestingly saw improved oxidative status at 4 days-post (i.e., 8-OHdG and 8-oxoGuo) and 7 days-post (i.e., 8-OHdG), lending evidence to the adaptive antioxidative effects following moderate dose acute exercise (Larsen et al., 2020). Rosa et al. compared biomarkers of redox balance between masters endurance and sprint athletes and reported that sprint athletes showed significantly lower baseline lipid peroxidation (i.e., F_2-Isoprostaines) levels, suggesting that event distance is an influential factor (Rosa et al., 2020). Collectively, these results support the hormetic theory, with a bi-phasic pattern where, beyond a minimum threshold, low to moderate-dose stress (relative to the athlete) is adaptive, with temporal shifts in oxidative balance and markers of oxidative damage (Radak et al., 2017), whereas high-dose acute stress shows a slower recovery pattern (Turner et al., 2014) and high chronic doses (with insufficient recovery) results in high baseline levels and a blunted oxidative response (Tanskanen et al., 2010). High exogenous anti-oxidant intake has also been shown to blunt the oxidative response (Rothschild & Bishop, 2020) and

should thus be considered when evaluating redox research, although the field of anti-oxidant research itself is expansive and beyond the purview of this literature review, however comprehensive reviews have been recently published by Rothschild and Bishop (Rothschild & Bishop, 2020), Pastor and Tur (Pastor & Tur, 2019) and Mason et al. (Mason et al., 2020).

Sex Differences

There is a paucity of published data on sex differences in redox biology (Di Florio et al., 2020) and due to the complexity of redox biochemistry the data that has been reported is equivocal as estrogens have been reported to have both antioxidant (Mendelsohn, 2002; Mendelsohn & Karas, 1999) and pro-oxidative effects (Liehr, 1996; Markides, Roy, & Liehr, 1998; Muzandu et al., 2005). Physiologically, female athletes have lover hemoglobin mass (Murphy, 2014) and correspondingly lower relative VO_2max values than male athletes (Diaz-Canestro & Montero, 2019), which would potentially attenuate the ROS generation during aerobic activities, yet due to the known oxidative effects of oral contraceptives and their widespread use (Cauci, Buligan, Marangone, & Francescato, 2016), if oral contraceptive use is not controlled for it would be expected to find elevated oxidative states in female athletes. Yet Nielsen et al. was one of the first to show differential exercise effects between male and female athletes (not controlling for contraceptive use) and female athletes actually showed improved redox status (i.e., a reduction in granulocyte derived ROS) post half-marathon, with no change in male subjects (Nielsen, Hagberg, & Lyberg, 2004). Additionally, they reported a post-marathon reduction in leukocyte ROS generation capacity and speculate that this reduced signaling capacity could explain the mechanism behind the hypothesis that athletes are more susceptible to infectious pathogens immediately post intensive exercise (Nielsen et al., 2004).

Similarly, Bloomer and Fisher-Wellman evaluated the effect of sex, training status and dietary intake on baseline measures of oxidative stress (i.e., plasm PC, MDA and 8-OHdG) and found that female participants had significantly lower oxidative stress (i.e., MDA values) than male participants, trained participants had significantly lower PC and MDA values than untrained participants, and there was an additive effect with trained women having the lowest baseline MDA values (Bloomer & Fisher-Wellman, 2008). There was no significant difference noted between any groups and 8-OHdG and only a weak correlation between protein (positive) and vitamin C (negative) intake and MDA, primarily in trained men. It is of note in the context of redox in elite athletes that the criteria used to categorize individuals as "trained" was the participation in structured exercise for a minimum of 3 hours per week for at least 6 months before the study (Bloomer & Fisher-Wellman, 2008), which is exiguous relative to well-trained and elite athletes. However, and a study of professional triathletes who trained 9-15 hours per week that measured oxidative DNA damage via 8-OHdG at the start and end of the racing season also reported no significant difference between male and female athletes (Zainudin et al., 2019). Another study, which evaluated sex differences in adolescent athletes (swimmers) found homogeneity of redox biomarkers between male and female athletes (Kabasakalis et al., 2009). Finally, and most applicably, Lewis et al. performed a study of the effect of acute exercise using both elite male and female endurance athletes and found no effect of sex after either submaximal or maximal exercise bouts in (Lewis, Towey, et al., 2016).

Analytically, Jones et al. analyzed 26 common chemical and hematological tests for sex, age and time between sample collection influences for within-subjects variation and reported a lack of effect of sex, age or time between samples for the analytes tested (G. R. D. Jones, 2019). However, analyses by Carobene et al. of the effect of sex on the within-subject

variation of 9 enzyme biomarkers in serum and reported lower between-subject variation in women for creatine kinase (CK), alanine amino transferase (ALT) and γ-glutamyl transferase (GGT), but no significant sex difference in within-subject variation of any biomarker tested (Carobene, Roraas, et al., 2017). A separate study of serum Creatine by Carobene et al. showed no between-subject or within-subject differences between male and female subject, yet mean serum creatine values were significantly higher in males (Carobene, Marino, et al., 2017). Further study of sex differences in 15 common protein biomarkers by Carobene et al. showed statistically different mean concentration values as well as between-subject variation for the majority of biomarkers tested, indicating a need for sex specific biological variation estimates (Carobene et al., 2019). Together these results indicate that that effect of sex on biomarker mean, within-subject and between-subject values is biomarker specific and likely population specific (Aarsand et al., 2018). There is limited mean, within-subject, between-subject and athlete specific data on redox biomarkers, but Lewis et al. did report similar within-subject variation for the redox biomarkers Free Radical Oxidation Test (FORT) and Free Radical Oxidation Defense (FORD) in athletes, though no assessment of variation by sex was reported as the testing only included male athletes (Lewis, Newell, et al., 2016).

Redox in Elite Sport

Oxidative stress in elite athletes has been evaluated across several sports and have been found to vary across sport disciplines (Arsic et al., 2016; Cubrilo et al., 2011; Czuczejko, Sielski, Wozniak, Wozniak, & Szewczyk-Golec, 2019; Hadzovic-Dzuvo et al., 2014; Sohail et al., 2020). Elite athletes are a particularly good model for exercise induced oxidative stress due to consistently high-volume and well-controlled training. Elite athletes also tend to train

at or near physiological training limits and therefore may be particularly sensitive to acute changes in training load (Thorpe et al., 2017). Furthermore, due to minute performance differences at the top-level, active management of training load via objective physiological measures such as oxidative stress biomarkers have great utility (Pedlar et al., 2019). Redox studies with elite athletes include alpine ski racers (Schippinger et al., 2009; Subudhi, Davis, Kipp, & Askew, 2001), combat sports (Cubrilo et al., 2011; Dopsaj et al., 2013; Hadzovic-Dzuvo et al., 2014; Imai et al., 2002; Pesic et al., 2012; Volodchenko, Podrigalo, Iermakov, Zychowska, & Jagiello, 2019), weight lifters (J. F. Liu et al., 2005; Sohail et al., 2020), water polo players (Arsic et al., 2016; Varamenti et al., 2013; Vecchio et al., 2017), rowers (Cubrilo et al., 2011; Kurgan, Logan-Sprenger, Falk, & Klentrou, 2018; Lewis et al., 2018), handball players (Cikiriz et al., 2020), soccer players (Andersson, Karlsen, Blomhoff, Raastad, & Kadi, 2010; Arsic et al., 2016; Becatti et al., 2017; Cavarretta et al., 2018; Gonzalez-Garrido, Garcia-Sanchez, Garrido-Llanos, & Olivares-Corichi, 2017; Hadzovic-Dzuvo et al., 2014; Jakovljevic, Zlatkovic, Cubrilo, Pantic, & Djuric, 2011; Le Moal et al., 2016; Povoas et al., 2020; Silva et al., 2013; Siquier Coll et al., 2019; Sohail et al., 2020), field hockey players (Rosa-Lima, Lannes, Viana-Gomes, Pierucci, & Salerno, 2015), volleyball players (Martinovic et al., 2011), basketball players (Chang et al., 2007; Hadzovic-Dzuvo et al., 2014; Spanidis et al., 2016; Zembron-Lacny, Slowinska-Lisowska, & Ziemba, 2010), triathletes (Garcia-Flores et al., 2016; Zainudin et al., 2019), swimmers (Leon-Lopez et al., 2018), cyclists (Cubrilo et al., 2011; Leonardo-Mendonca et al., 2014; Sohail et al., 2020), and runners (Koivisto et al., 2019; Lewis, Towey, et al., 2016; Michalickova et al., 2020; Sohail et al., 2020; Tong et al., 2016; Vezzoli et al., 2016).

The greater the relative exercise stimulus, the more prominent the shifts in oxidative balance, therefore multi-day (i.e., semi-acute) training camps are often the platform for studying training related alterations of redox status in elite sports due to the highly developed capacity for training of elite athletes. An early study of oxidative stress and antioxidant status by Subudhi et al. on 12 members of the USA Alpine Ski Team over the course of an intensive 10-Day off-season (i.e., summer) training camp showed an acute rise in antioxidant capacity [i.e., Trolox® equivalent antioxidant capacity (TEAC) and uric acid (UA)] with a concomitant reduction in plasma antioxidant values [i.e., α-tocopherol, γ-tocopherol, GSH, and superoxide dismutase (SOD)], indicating that the antioxidants may have been utilized to counteract the oxidative stress of the acute rise in training load (Subudhi et al., 2001). The authors concluded that the lack of increase of oxidative stress suggests adequate antioxidant capacity of the athletes relative to the training stimulus of the 10-day camp but the decrease in plasma antioxidants α- and γ-tocopherol suggests that the capacity may have been exceeded over a longer period of training.

A subsequent study of oxidative stress by Imai et al. evaluated the change in redox state of elite martial arts (kendo) athletes after an intensive 5-day off-season training camp and reported an increase in oxidative state [i.e., oxidized human serum albumen (HSA)], which was not seen during normal training (Imai et al., 2002). Martinovic et al. monitored oxidative stress biomarkers in elite female volleyball athletes over a 6-week pre-competition period to compare redox status of antioxidant supplemented versus non-supplemented athletes and found that the supplementation increased antioxidative defense [i.e., SOD activity and Biological Antioxidative Potential (BAP)] and reduced ROS (i.e., superoxide anion) and in

supplemented volleyball athletes, whereas no changes in these markers were seen in the control (i.e., non-supplemented) group (Martinovic et al., 2011).

Altitude Training. Altitude training is both a requisite part of elite endurance sport due to the potential of increasing hemoglobin mass and associate maximal oxygen uptake (VO_2max) and performance (Sitkowski et al., 2019) yet trying to balance the additional physiological stress associated of hypoxia with training load often results in mixed individual outcomes (i.e., "responders" vs "non-responders") (Rusko, Tikkanen, & Peltonen, 2004). And because hypoxia is known to increase oxidative stress (Quindry, Dumke, Slivka, & Ruby, 2016), the oxidative stress and antioxidative capacity of athletes during altitude training camps are an attractive area of research for elite sport. In a study of elite middle distance male runners by Pialoux et al., they showed that athletes who were subjected to 18 days of Live-High Train-Low (LHTL) simulated altitude training (i.e., 2500-3000m simulated living altitude, 1200m training altitude) had decreased antioxidative status (i.e., FRAP, α-tocopherol, β-carotene and lycopene) relative to controls who lived and trained at 1200m (Pialoux, Mounier, Rock, Mazur, Schmitt, Richalet, Robach, Brugniaux, et al., 2009). However, after being challenged with an acute hypoxic test (i.e., 10 minutes at 4800m simulated altitude), the control group showed increased oxidative damage (i.e., MDA and AOPP), whereas there was no effect on the LHTL group (Pialoux, Mounier, Rock, Mazur, Schmitt, Richalet, Robach, Brugniaux, et al., 2009). In a follow-up study of elite cross-country skiers by Pialoux, et al, there was a significant increase in oxidative damage (i.e., AOPP) as well as reduction in antioxidative capacity (FRAP, TEAC, lycopene, and β-carotene) immediately post 18-day natural altitude training camp (Pialoux et al., 2010). Strikingly, at 14-days post altitude camp, the AOPP and TEAC had returned to baseline, but the FRAP, lycopene, and β-carotene were all still significantly

reduced relative to pre-altitude values (Pialoux et al., 2010). An altitude camp study of elite triathletes by Garcia-Flores et al. found that training at altitude over a 2-week period showed increased oxidative damage (i.e., F4-NeuroPs and F2-dihomo-isoPs) which was not observed after the same training was completed at sea level (Garcia-Flores et al., 2016). Similarly, a subsequent study by Leon-Lopez et al. which evaluated the oxidative stress of elite swimmers after a 3-4 week altitude training camp reported increased oxidated stress (i.e., MDA and PC) and decreased antioxidative capacity (i.e., GPx and GSSG/GSH ratio) supporting the work by Garcia-Flores (Leon-Lopez et al., 2018). Interestingly, relative to the other altitude trained groups and sea level controls, the athletes using a live high, train low (LHTL) approach had both more favorable antioxidant status in this study as well as showed the greatest improvement in performance in this group's previous research (Rodriguez et al., 2015). Koivisto et al. monitored the effect of increasing antioxidant rich foods in elite endurance athletes (i.e., swimmers, rowers, kayakers, triathlete and middle distance runner) during a 3-week altitude training camp and increases in oxidative damage (i.e., 8-epi-PGF2α) and inflammatory cytokine IL-7 (Nosalski & Guzik, 2021) in both supplemented and non-supplemented groups (Koivisto et al., 2019). They also found that athletes supplemented with increased antioxidant food intake exhibited both elevated antioxidant capacity [i.e., Ferric Reducing Ability of Plasma (FRAP)] and reduced airway inflammatory cytokine IL-13 (Le Floc'h et al., 2020) relative to the non-supplemented group (Koivisto et al., 2019). In a sister publication, it was also noted that both groups showed increased hemoglobin mass and VO_2max, indicating the effectiveness of the altitude camp, although there were no differences in hemoglobin mass, VO_2max or performance between the supplemented and non-supplemented groups (Koivisto et al., 2018). In the context of altitude training camps, it is also

of note that simulated altitude exposure of as little as one hour at very high altitude (4800m) and three hours at high altitude (3000m) were sufficient to show oxidative damage [i.e., increases in MDA and Advanced Oxidative Protein Products (AOPP)] as well as reductions in antioxidative capacity [i.e., FRAP (3000m only) and plasma α-tocopherol] in elite Nordic skiers, swimmers and track athletes (Pialoux, Mounier, Rock, Mazur, Schmitt, Richalet, Robach, Coudert, et al., 2009).

Seasonal Variation. Seasonal changes of oxidative stress in elite athletes is another area of research interest as understanding the influence of time-in-season is requisite for proper management of athletes training load (Varamenti et al., 2013). A study of eight professional American football players by Schippinger et al. was the first to report alterations in redox balance in an elite athlete population (Schippinger et al., 2002). This study evaluated four timepoints, one pre-season timepoint (i.e., March) and three timepoints during the competitive season (i.e., May, June, July) and reported significant elevations in oxidation (i.e., serum peroxide concentrations and aAB against oxidized LDL) during the season, relative to pre-season values. In addition, they found increases in serum ascorbate concentration during the season, relative to pre-season values, with no elevation in β-Carotene, α- tocopherol or γ-tocopherol. Notable individual differences were also reported as four of the eight athletes studied showed increased in peroxide concentrations that were several times baseline (pre-season) values, whereas the other four athletes showed no increase. Because alterations in redox balance could not be predicted from baseline values, yet significantly increased in some athletes during the competitive season, this seminal work concluded that oxidative stress should be monitored in all athletes (Schippinger et al., 2002). Another early investigation of oxidative stress across a season of professional rugby players was performed by Finaud et al.

and showed an increase in oxidative stress (Rmax) along with a decrease in antioxidative capacity [i.e., Lag Phase (LP) & α-tocopherol, but not TAC] that corresponded with periods of high training load (i.e., during the beginning of the competitive period of the season (i.e., December)), relative to the beginning of the season (i.e., September) and prior to playoffs (i.e., April) (Finaud et al., 2006). Another investigation of oxidative stress across a season of elite athlete training by Schippinger et al. revealed that time-in-season also had a significant effect on oxidative stress in elite male alpine skiers (Schippinger et al., 2009). Pre-season (i.e., June), and the first three months of the competitive season (i.e., Nov, Dec, Jan) were collected prior to competitive events and showed a significant increase in oxidative stress in November (i.e., total hydroperoxides) and December (i.e., aAb against oxidized LDL) with no change in antioxidant levels (i.e., α- and γ-tocopherol, β-carotene, ascorbic acid and lag time of DPHPC degradation) (Schippinger et al., 2009). Most notably, the authors identified for the first time an association between elite athlete performance and oxidative stress, as the athletes with the lowest rise in total hydroperoxides had the best performance at the November competition (Schippinger et al., 2009). Work by Pesic et al. comparing the seasonal periodization Preparatory Period versus the Competition Period of elite karate athletes showed decreased baseline antioxidative activity (i.e., CAT) in the Competition Period (after 3 months of training) (Pesic et al., 2012). However, when challenged with a single regular karate training session in the Competition Period, athletes showed an increase in antioxidative potential (i.e., CAT) post workout and therefore the authors concluded that although baseline CAT was reduced, the adequate response of the antioxidant system of the athletes to acute exercise remained preserved (Pesic et al., 2012). A study of elite female water polo players throughout a season by Varamenti et al. showed a reduction of antioxidative capacity (i.e., TAC) two

months into the season but did not see an increase in oxidative stress until 5 months into the season, which continued through 7 months [i.e., TBARS & PC (7 months only)] (Varamenti et al., 2013). Of note, the final sample at 7 months was of tapered (i.e., rested) athletes two days before the start of playoffs, which seemed to recover antioxidative capacity (i.e., TAC), but did not reduce oxidative stress (i.e., TBARS & PC) (Varamenti et al., 2013). Similarly, work by Silva et al. showed elevated oxidative stress (i.e., TBARS & PC) in professional soccer players mid and late-season, relative to pre- and post-season values (Silva et al., 2014). Antioxidative capacity (i.e., TAS) was also assessed, with no significant change across the season (Silva et al., 2014). Similarly, another study by Moal et al. showed a decrease in antioxidative capacity (i.e., GSH/GSSG ratio) during periods of higher training load for professional soccer players (i.e., mid- and championship seasons relative to pre- and post-season), although no significant change in plasma antioxidant (i.e., α-tocopherol, β-carotene and retinol) or antioxidative enzyme (i.e., GPx or SOD) levels were detected (Le Moal et al., 2016). Becatti et al. found similar results with elevated oxidative stress values (i.e., TBARS & PC) in-season relative to baseline (pre-training) values (Becatti et al., 2017), yet antioxidative capacity (i.e., TAC & GSH/GSSG ratio) were significantly reduced at the beginning of the season, but then recovered to pre-training values or higher by mid-season and later, potentially highlighting adaptation of the athletes to the training (Becatti et al., 2017). Additional work showing positive oxidative adaptations to training with nationally and internationally competitive middle- and long-distance runners by Munoz Marin, et al, showed that although plasma concentrations of some antioxidant vitamins (i.e., vitamin C and vitamin A, but not vitamin E) decreased during the season relative to pre-season values, markers of oxidative lipid damage (i.e., PUFA/SFA ratios, but not MDA) also decreased (Munoz Marin

et al., 2018). This led the authors to conclude that the exercise-induced oxidative stress from regular training throughout the season caused an adaptive response promoting endogenous antioxidative capacity of the athletes (Munoz Marin et al., 2018), however no measures of antioxidative capacity were included. In addition, this study used a combination of competitive cross-country and track athletes, which have different competitive seasons and therefore may not have obtained optimal resolution in terms identifying the seasonal variation of either sub-set.

Point-of-Care Biomarkers. There has been pervasive research over the last few decades evaluating oxidative stress from recreational to elite athletes which has been instrumental in discovering the value of oxidative stress biomarkers in sport. But due to the constraints of analytic methods (i.e., requirement for remote labs, lengthy processing time and invasive venous blood sampling), application has been limited to retrospective analysis. The recent development of X-band EPR (Bruker Corporation, Billerica, MA) took the applied potential of oxidative stress in athletes a step forward with the ability to assess oxidative stress less invasively, with only the requirement of capillary blood, however the large equipment necessary to analyze the samples still requires a remote lab still limiting prospective use. However, with the introduction of capillary blood oxidative stress testing via portable absorbance analyzers (Callegari 1930, Parma Italy), oxidative balance can now be assessed minimally invasive, near real-time and at the point-of-care (e.g., on the field), opening the possibility of prospective use (Lewis, Newell, et al., 2016). These systems provide both a snapshot of both blood plasma pro-oxidative state via the Free Radical Oxygen Test (i.e., FORT) and antioxidative capacity via Free Radical Oxygen Defense (i.e., FORD) test, which can be evaluated individually or together as a composite index of oxidative stress (i.e., OSI)

(Lewis, Towey, et al., 2016). The first use of these PoC biomarkers of oxidative stress in athletes was by Lewis et al, where they were shown to be reliable analytical measures in elite athletes with a low analytical coefficient of variation (CVa) of 3.9% (FORT) and 3.7% (FORD) (Lewis, Newell, et al., 2016). Additionally these assays were evaluated in a separate group of well-trained athletes for biological variability (BV) (i.e., coefficient of within-subjects (CVw) and between-subjects variation (CVb)), Critical Difference Values (CDV), Index of Individuality (II) and circadian variation in which collectively determined these biomarkers to be reliable measures with low within-subjects variation (CVw FORT, 5.0%; CVw FORD 7.5%), but due to the higher between-subjects variation (CVb FORT, 17.3%; CVb FORD, 9.6%), general lab reference ranges would be of limited utility an therefore characterization of values across different sample populations and individuals is warranted (Lewis, Newell, et al., 2016). In follow-up work by Lewis et al. which evaluated the oxidative stress response of elite runners and triathletes to an acute exercise bout, a rise in peripheral blood oxidation levels was identified (i.e., FORT) after a maximal treadmill test, but not after a sub-max test (Lewis, Towey, et al., 2016). Furthermore, they also identified a corresponding rise in antioxidant activity (i.e., FORD) after both the maximal and sub-max tests, which resulted in an overall reduced composite oxidative stress (OS) score (Lewis, Towey, et al., 2016). Subsequent research by this group with Olympic rowers demonstrated for the first time a significant association between greater antioxidative capacity (i.e., FORD) and lower illness rates (Lewis et al., 2020). In addition, lower (i.e., more favorable) composite Oxidative Stress Index (i.e., OSI) values were associated with both lower injury and illness in their cohort of elite rowers (Lewis et al., 2020). Other work by Quinn et al. not only showed FORT and FORD biomarkers to be responsive to an acute exercise bout, but also showed reliability of response across

multiple trials (Quinn et al., 2020). In addition, they reported for the first time that these measures correlated well with the historical, lab-based oxidative stress measures of F2-isoprostanes and Total Anti-oxidative Capacity (TAC), with FORD and TAC showing the strongest correlation (Quinn et al., 2020). Most recently, work by Mckay et al. with Collegiate American football players showed significant associations between FORT, FORD and OSI with countermovement jump performance metrics (i.e., concentric impulse, flight time and reactive strength index modified (RSImod)), urinary cortisol and the standard subjective feedback measures of fatigue, sleep and soreness (McKay et al., 2021).

Summary

Training induced oxidative stress in elite sport is a balancing act as pro-oxidative processes are integral to signaling pathways required for the adaptive process to exercise, yet if too great then oxidative damage occurs putting athletes at risk of underperformance, injury and illness. Biomarkers of oxidative stress and oxidative damage have been applied to exercise and elite sport for several decades and these studies have been instrumental in elucidating the mechanisms and value of oxidative stress biomarkers in sport. But due to the constraints of analytic methods (i.e., remote labs, lengthy processing time and invasive venous blood sampling), application of these biomarkers is limited to retrospective analysis. However, due to more recent advances in technology and application, blood plasma oxidative balance can now be assessed near real-time at the point-of-care (PoC) with only the need for a capillary blood sample, creating the potential for use as a prospective measure that could be used to more tightly regulate training application.

But work to determine if these PoC biomarkers can be reliably applied as objective measures for both heath and exercise is still emerging. Early assay validation work showed FORT to be a reliable test method in both clinical groups (i.e., diabetic patients and patients with hypercholesterolemia) and healthy controls (Garelnabi, Brown, & Le, 2008), and more recent work by Lewis et al. showing FORT and FORD to be reliable analytical measures in athletes with both low analytical and within-subjects variation (Lewis, Newell, et al., 2016), as well as responsive to acute exercise (Lewis, Towey, et al., 2016). Subsequent work by Quinn et al. not only showed these PoC biomarkers of oxidative stress to be responsive to acute exercise, but also showed reliability of response across multiple trials and a strong correlation with historical, lab-based oxidative stress measures (Quinn et al., 2020). And recent work has shown these PoC measures to be directly associated with illness and injury in Olympic rowers (Lewis et al., 2020), in addition to significantly correlate with the standard subjective measures of soreness and fatigue, which are the primary measures currently used to manage athlete training load (Bolling et al., 2020; Saw et al., 2016). However, to date there is little known about the effectiveness of these PoC measures in elite endurance track athletes, the clinical relevance of values seen in athletes, variations across a season or the effect of the semi-acute physiological stressors of vaccination and altitude exposure.

Aims and Hypotheses

Specific Aims

The overarching aim of this research was to determine how different conditions affect PoC biomarkers of oxidative stress in elite endurance track athletes and compare those values with disease populations and sedentary controls. The overarching hypothesis was that elite endurance track athletes will display increased oxidative stress levels in response to semi-acute physiological stressors (e.g., training phase, altitude exposure, vaccination) yet will exhibit significantly lower mean values than disease populations, but not sedentary controls. It was anticipated that this work will provide additional support for the use of PoC oxidative stress as a suitable objective biomarker to monitor the physiological condition of athletes to aid in training load management and help reduce the risk of underperformance, illness, and injury.

AIMS & Hypotheses

AIM 1: Compare PoC biomarkers of oxidative stress in elite endurance track athletes with published data for disease states and sedentary controls.

Hypothesis 1*: PoC biomarkers of oxidative stress in Elite endurance track athletes will be significantly lower than published values associated with disease states but not sedentary controls.*

AIM 2: Investigate alterations in the redox balance of elite endurance track athletes over the course of the competitive year using PoC biomarkers of oxidative stress.

Hypothesis 2*: PoC biomarkers of oxidative stress will be significantly higher in the pre-season months (i.e., December-March), relative to other months of the year.*

AIM 3: Determine if high altitude exposure effects PoC biomarkers of oxidative stress in elite endurance track athletes.

Hypothesis 3: *Periods of altitude training will be associated with significantly higher oxidative stress than periods of sea level training in elite endurance track athletes.*

Exploratory AIM: Determine if there is a change in PoC biomarkers of oxidative stress after mRNA COVID-19 vaccination of elite endurance track athletes.

Explor*atory Hypothesis**: mRNA (mRNA-1273) COVID-19 vaccination will be associated with a significant but transient increase of oxidative stress in elite endurance track athletes.*

Chapter 2

Materials and Methods

Study Purpose and Approach

The purpose of this study was to determine how different conditions affect the redox balance of elite endurance track athletes using PoC biomarkers of oxidative stress and to compare those values with published values for disease populations and sedentary controls. The primary objective of the data collected was to improve the health and performance of the athletes, therefore the goal of this research was to evaluate changes in oxidative status with the intent to publish supporting information to allow for continued development of PoC blood biomarkers of oxidative stress as an objective measure of physiological balance to assist in the management of health and performance.

The research design of this study was a longitudinal, retrospective observational study of elite athletes over the course of multiple seasons of training and racing. The inclusion criteria were that the athletes must be competitive at the USA National level in one or more endurance track/running events. The rationale for selection of this population was risk of underperformance, illness and injury, scarcity of data in this population, and consistency of training approach and environmental conditions across athletes.

Redox data was collected from 24 elite endurance track athletes, with event distances from 800 meters to the Marathon. Endurance athletes have been defined as any athletes with competition performance that exceeds 60 seconds (Lewis et al., 2015). Athletes were all free living, engaged in group training with training managed under a single, uniform coaching

system. Athletes were also subject to United States (USADA) antidoping standards and oxidative testing was performed through all phases of training. This secondary data analysis study was approved by Orreco and the University of Houston via data use agreement obtained from both parties as well as University of Houston Institutional Review Board (IRB) approval (IRB ID: STUDY00003712).

Analytical methods and Data acquisition

All redox and hsCRP data was collected by Orreco (Teddington, UK) from March 2016 to June 2021 from 24 elite endurance track athletes, using PoC biomarkers of oxidative stress. Oxidative testing was performed year-around via whole blood capillary samples taken from each ear lobe (duplicate samples) and immediately processed at room temperature per manufacturer's instructions (CR3000, Callegari1930, Catellani Group, Parma, Italy). Samples were primarily collected between 6AM and 10AM with athletes in a hydrated, fasted and rested state, though concessions were made where necessary to minimize training disruption. In addition to hsCRP, two redox test methods were completed, which included the Free Radical Oxidation Test (FORT) and Free Oxygen Radical Defense (FORD) tests. In addition, the Oxidative Stress (OS) index was calculated from the FORT and FORD test values (OSI = FORT/FORD). These analytical methods were chosen because they have been shown to have good analytical validity, with inter-assay coefficients of variation for FORT and FORD of <5% and 7%, respectively (Lewis, Newell, et al., 2016). The assays have been independently validated for sample-to-sample reliability as well as accuracy against standard laboratory measures of F2-isoprostaines and total antioxidant capacity (TOC) (Quinn et al., 2020). In addition, this test method is a portable (i.e., PoC) method that is minimally invasive (i.e., requires only 70µl of capillary blood) and provides rapid results (approximately 10 minutes).

Samples were collected and analyzed by trained personnel with strict adherence to Manufacturers' quality assurance protocols.

Statistical Approach: (Statistical Power and Analysis)

Analysis was performed using SPSS version 27 or later (IBM, Chicago, IL, USA). Statistical tests were described in detail within the *Analysis* section below but included restricted maximum likelihood linear mixed models as the primary tool due to the repeated measures and unequal sample sizes. Briefly, separate analyses of measures of pro-oxidant status (FORT), antioxidative capacity (FORD) oxidative stress balance (OSI) and hsCRP as the dependent variable were performed, with main effects for the grouping variable as a fixed effect, and athlete entered as a random effect. Additional analysis and methods were utilized as needed to answer each of the research questions and statistical significance was set at $p<0.05$.

Specific Aim 1: Compare PoC biomarkers of oxidative stress in elite endurance track athletes with published data for disease states and sedentary controls.

Redox values from 24 elite endurance track athletes (16 male and 8 female) sampled across the annual training cycle from 2016 to 2021 were used for this analysis. There were total of 1478 samples used for analysis with a single average FORT and FORD value used for each athlete and then a sample mean and standard deviation for the 24 athletes was calculated from those values. The athlete sample was also analyzed by sex. Because a significant sex difference was found, the sample was split by sex and the sex with the least favorable redox balance (i.e., females) was used for comparison with clinical (i.e., disease state) samples using the identical FORT and FORD analysis via summary independent samples T-Test of the

difference between observed means (Altman, 1991). Equal variance was not assumed and "Exact" (not asymptotic) 95% confidence intervals for the difference was used due to smaller sample sizes and for the most conservative estimate. Sedentary controls from the studies of PoC oxidative stress in disease populations was pooled to reduce the potential of type II errors and Bonferroni correction as post hoc analysis was used to control for family-wise type I errors (Aickin & Gensler, 1996; H. C. Liu, Chaou, Lo, Chung, & Hwang, 2022).

Hypothesis 1: PoC biomarkers of oxidative stress in elite endurance track athletes will be significantly lower than published values associated with disease states but not sedentary controls.

Specific Aim 2: Investigate alterations in the redox balance of elite endurance track athletes over the course of the competitive year using PoC biomarkers of oxidative stress.

Redox samples collected by Orreco for the 24 elite endurance track athletes over a 5-year period was used for this analysis. To weight athletes equally, only one average value per analysis period (i.e., month) was used for each athlete. If multiple seasons of data exist for an athlete, they were averaged into one annual training cycle as not enough data existed to perform an analysis across training years. The annual training cycle was categorized by general training phase (Preparatory, Competitive, and Transition), sub-category [General Preparation (pre-season), Specific Preparation (indoor season), Pre-Competition (outdoor season), Competition (National and World Finals) and Transition (off-season)] and month. There were total of 1478 samples (378 averaged athlete months) used for this analysis. For the experimental design, the PoC oxidative stress biomarkers of FORD, FORT and OSI were analyzed via Restricted Maximum Likelihood Linear Mixed Model with Satterthwaite small sample approximation for

degrees of freedom (df). Three separate models were used, one for each dependent variable of FORD, FORT and OSI. Each Model included main effects for *Period* (e.g., month) as a fixed effect and used *Athlete* as a random effect with a pairwise post-hoc comparison of main effects using Bonferroni correction. Additional models were used to evaluate differences in OSI by general training periodization phases (i.e., Preparatory, Competition, and Transition) as well as sub phases (i.e., General Preparatory, Specific Preparatory, Pre-Competition, Competition and Transition). The influence of *Sex* was also evaluated in an exploratory, descriptive analysis as sex differences have been previously reported although results are equivocal. To evaluate sex differences, three additional Restricted Maximum Likelihood Linear Mixed Models with Satterthwaite approximation were used, one for each dependent variable of FORD, FORT and OSI. Each Model included main effects for *Month* and *Sex* and the interactions of *Month* by *Sex* as fixed effects and used *Athlete* as a random effect with a pairwise post-hoc comparison of main effects using Bonferroni correction.

Hypothesis 2: PoC biomarkers of oxidative stress will be significantly higher in the pre-season training months (i.e., December-March), relative to other months of the year.

Specific Aim 3: Determine if high altitude exposure effects PoC biomarkers of oxidative stress in elite endurance track athletes.

The Redox samples collected for the 8 elite endurance track athletes throughout the course of an altitude camp were used for this analysis. The altitude camp was 31 days in duration and performed at a base altitude of approximately 2100 meters. A total of 388 samples were used for this analysis with 77 data points used for statistical analysis after individual sea level baseline values were calculated for each athlete. Four altitude samples were taken for each

athlete (on altitude day 5, 12, 19 and 26), except for two athletes who only had complete samples for three altitude timepoints. For the experimental design, the PoC oxidative stress biomarkers were analyzed via Restricted Maximum Likelihood Linear Mixed Model with Satterthwaite small sample df approximation. Five timepoints were entered into the model, including a calculated baseline sea level timepoint and the four sequential altitude timepoints. The sea level datapoint was calculated as the mean FORT, FORD and OSI value at sea level for each athlete as sea level data immediately preceding the altitude camp was not available. Three separate models were used, one for each dependent variable of FORD, FORT and OSI. Each Model included main effects for *Timepoint* as a fixed effect and used *Athlete* as a random effect with a pairwise post-hoc comparison of main effects using Bonferroni correction.

Hypothesis 3: Periods of altitude training will be associated with significantly higher oxidative stress than periods of sea level training in elite endurance track athletes.

Exploratory AIM: Determine if there is a change in PoC biomarkers of oxidative stress after mRNA COVID-19 vaccination of elite endurance track athletes.

And as an exploratory AIM, the oxidative stress associated with COVID-19 vaccination was evaluated. Redox samples were collected by Orreco for 9 elite endurance track athletes before, during (between vaccine shots 1 and 2) and after vaccination to monitor changes in oxidative status. All athletes received the Moderna mRNA-1273 (SPIKEVAX™, Moderna Co., Cambridge, MA, USA) vaccine in March and April of 2021 with an inter-dose interval of 25-33 days with an average of 28 days, which is the target prescribed interval. The date the 1st dose was administered was considered day zero and the 2nd dose was administered on day 28±3. Athletes typically arrived at altitude two days before the first vaccine dose and returned

to sea level 3-6 days after administration of the second vaccine dose. Assigned athlete ID numbers 3, 5, 6 and 7 are the corresponding athletes analyzed for the impact of altitude on redox balance. A total of approximately 306 samples were used for this analysis, with 208 oxidative stress (i.e., FORT & FORD) and 98 high sensitivity C-Reactive Protein (hsCRP). The 9-week period immediately preceding vaccination was used as a *Pre-Vaccine baseline* for each athlete. Post vaccine sampling was conducted approximately weekly for 4 *Inter-Dose* samples, with all 4 samples collected within 25-33 days of the first vaccine dose and two *Post-Vaccination* samples were collected within 14-24 days after completing the two dose vaccine series. For the experimental design, the PoC oxidative stress biomarkers of FORD, FORT, OSI and hsCRP were analyzed via Restricted Maximum Likelihood Linear Mixed Model with Satterthwaite small sample df approximation. Seven timepoints were used in the analysis for the independent variable of *Timepoint* (i.e., one averaged *Pre-Vaccine*, four *Inter-Dose* and two *Post-Vaccination* samples as described above. Four separate models were used, one for each dependent variable of FORD, FORT, OSI and hsCRP. Each Model included main effects for *Timepoint* as a fixed effect and *Athlete* as a random effect with pairwise post-hoc comparison of main effects using least significant difference (LSD).

Exploratory Hypothesis: mRNA (mRNA-1273) COVID-19 vaccination will be associated with a significant but transient increase of oxidative stress in elite endurance track athletes.

Strengths & Limitations:

Strengths: The subject population for this study is quite rare as access to elite athletes for scientific study is limited. Therefore, a longitudinal dataset of 24 World Class athletes over 5 years, with nearly 1500 discrete test samples is a very strong and rare dataset to analyze. An

additional strength is the homologous nature of this group of athletes who are all in the same location and managed under the same training system and therefore had similar external factors, as well as compete at approximately the same level (i.e., Internationally Competitive). Another marked strength is that this group of athletes contained eight elite female athletes, which are highly underserved in the literature, especially in terms of oxidative stress indices.

Limitations: First, this research proposes to compare oxidative stress values of this athlete sample to those in the literature to gauge clinical significance, and although sampling and analysis should be uniform as there is only one company producing these analysis kits, there was no way to verify the accuracy of these values other than though replication of results across multiple studies.

Second, because the population being evaluated were elite athletes, competitive at the highest level, control over sampling uniformity was limited, both in terms of athlete state and timing of samples. There was a concerted effort to perform sampling in the AM, before workouts, with the athletes fasted, rested and hydrated, but ultimately the athlete's training schedule took priority. In addition, there was variability in the time interval between repeated samples from different athletes.

Third, it was the hope that this work would contribute unique data and analysis to the literature so that this objective measure can be used to reduce injury and illness in athletes, while maintaining or increasing performance, however this study did not evaluate those outcomes directly.

Statistical Power: A pre-existing dataset from a homogenic sample population of 24 elite level (i.e., professional), endurance (i.e., 800m to Marathon event distance), internationally competitive track athletes (i.e., runners) was used for this study. Nearly 1500 discrete samples of FORT and FORD, as well as more than 700 composite OS scores over a 5-year period (March 2016 to June 2021) were available for analysis. Due to the retrospective analysis of this pre-existing dataset, no power analysis was performed.

Chapter 3

Results

Oxidative stress in elite endurance track athletes is lower than disease populations but not healthy controls

Redox samples from 293 subjects (106 male and 187 female) and 110 controls (35 male and 75 female) were available for comparison to our sample of 24 elite endurance track athletes (16 male and 8 female). To account for variation in number of samples per athlete, summary data for the elite endurance track athletes was calculated from the grand mean for each athlete The demographics of the independent sample groups used for comparison are listed in Table 1. Descriptors for control groups are verbatim from the associated research paper and age and BMI were included where available as both have been reported to affect bloodborne biomarkers of oxidative stress (M. A. Gaman, Epingeac, Diaconu, & Gaman, 2020; Skibska & Goraca, 2015).

Redox balance across independent sample groups is shown in Figure 1. Elite UK endurance athletes had the most favorable redox balance and the obese T2 diabetic and sickle cell disease samples had the least favorable. Statistical analysis revealed mean FORT values for all disease samples and women on oral contraceptives (WomenOC) were significantly higher than male and female elite endurance track athletes (EETA). FORD values for all disease samples and WomenOC were significantly lower than male and female EETA. In addition, FORD values for elite UK endurance athletes were significantly higher than male

and female EETA. There were no other significant differences detected for either FORT or FORD between the controls, active or athlete groups and EETA. Statistical comparisons between groups are detailed in Table 2.

Table 1. Demographics of independent sample groups used for comparison. Data are mean ±SD.

Sample Group	n	Female	Age	BMI	Reference
Type 1 Diabetic "w/some regular aerobic exercise"	8	4	49.1±10.5	25.0±3.2	(Francescato, Stel, Geat, & Cauci, 2014)
Type 2 (T2) Diabetic Women	22	22	55.1±11.8*	29.3±5.7*	(Pavlatou, Papastamataki, Apostolakou, Papassotiriou, & Tentolouris, 2009)
Type 2 (T2) Diabetic Men	23	N/A			
Obese T2 Diabetic	33	27	66.5±9.8	36.7±4.0	(M. A. Gaman et al., 2020)
Obese Non-Diabetic	69	54	60.1±9.8	35.3±3.6	
Iron Deficiency Anemia Females[1]	33	33	N/Av	N/Av	(Yoo et al., 2009)
Sickle Cell Disease (SCD)	40	24	28.9±13.6	N/Av	(Gizi et al., 2011)
Recreationally Active WomenOC[2]	8	8	24±5*	N/Av	(Quinn et al., 2020)
Recreationally Active Males	6	N/A		N/Av	
Well Trained Male Participants	12	0	30±7	23.7[#]	(Lewis, Newell, et al., 2016)
Elite UK Endurance Athletes	15	7	22±4	22.5[#]	
Elite Endurance Track Athletes	**24**	**8**	**N/Av**	**N/Av**	**N/A**
Controls	**n**	**Female**	**Age**	**BMI**	**Reference**
"Healthy Controls w/some regular aerobic exercise"	14	6	46.8±10.7	24.3±3.3	(Francescato et al., 2014)
"Apparently Healthy Controls"	10	10	N/Av	N/Av	(Pavlatou et al., 2009)
"Apparently Healthy Controls"	10	0	N/Av	N/Av	
"Healthy Individuals"	30	21	53.8±11.5	24.6±1.8	(M. A. Gaman et al., 2020)
"Healthy Controls"	21	21	N/Av	N/Av	(Yoo et al., 2009)
"Apparently Healthy volunteers"	25	17	26.4±10.3	N/Av	(Gizi et al., 2011)
Controls (Combined)	**110**	**75**	**N/Av**	**N/Av**	**N/A**

*Values from combined group of males and females. N/A indicates not applicable. N/Av indicates data not available.

[#]SD not available - calculated from aggregate height and weight values

[1] IDA defined as MCV <80 fL, hemoglobin concentration <12 g/dL, serum iron concentration <45 g/dL and serum ferritin <15ng/ml

[2] Women using combined, monophasic Oral Contraception

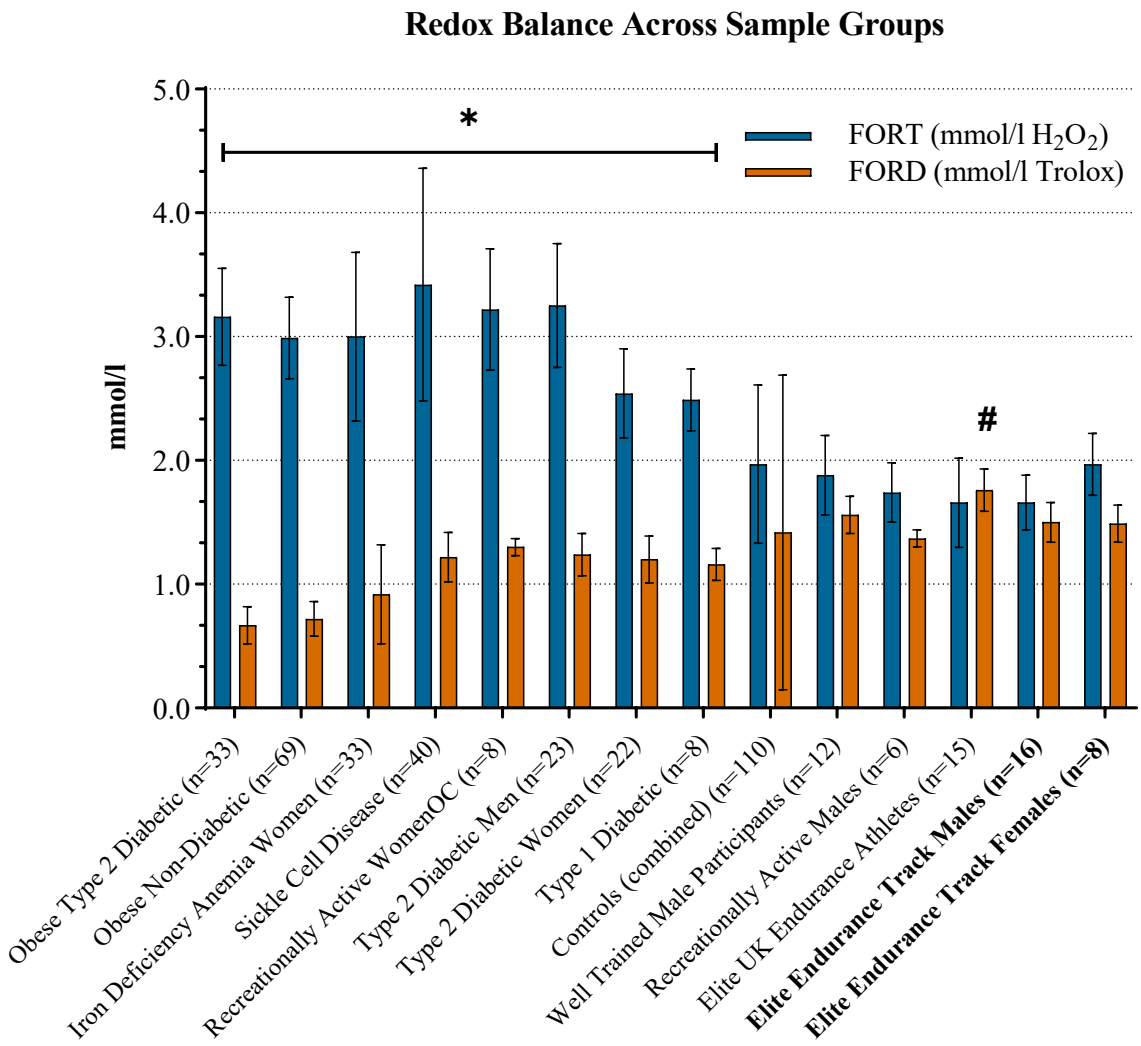

Figure 1. Redox balance across sample groups. Data are mean ±SD. Elite endurance track athlete data calculated from the grand mean for each athlete. * Indicates significantly higher FORT and lower FORD values relative to both male and female elite endurance track athletes. # Indicates significantly higher FORD value relative to both male and female elite endurance track athletes, $p<0.05$.

Table 2: Comparison of Elite Endurance Track Athletes with other Independent Sample Groups

Sample Group	Test	Mean	SD	Mean Diff.	SE Diff.	t-value	DF	95% CI	Sig.	Reference
Type 1 Diabetic	FORT	2.49	0.25	0.520	0.125	4.160	14.000	0.252 to 0.788	<.001*	(Francescato, Stel et al. 2014)
	FORD	1.16	0.13	-0.330	0.070	-4.702	13.723	-0.481 to -0.179	<.001*	
Type 2 (T2) Diabetic Women	FORT	2.54	0.36	0.570	0.117	4.869	18.105	0.324 to 0.816	<.001*	(Pavlatou, Papastamataki et al. 2009)
	FORD	1.20	0.19	-0.290	0.067	-4.346	15.762	-0.432 to -0.148	<.001*	
Type 2 (T2) Diabetic Men	FORT	3.25	0.50	1.280	0.137	9.365	24.771	0.998 to 1.562	<.001*	
	FORD	1.24	0.17	-0.250	0.064	-3.919	13.777	-0.378 to -0.113	0.002*	
Obese T2 Diabetic	FORT	3.16	0.39	1.190	0.120	9.908	19.435	0.939 to 1.441	<0.001*	(Gaman, Epingeac et al. 2020)
	FORD	0.67	0.15	-.0820	0.059	-13.872	10.668	-.0951 to -0.689	<0.001*	
Obese Non-Diabetic	FORT	2.99	0.33	1.020	0.097	10.526	10.072	0.804 to 1.236	<0.001*	
	FORD	0.72	0.14	-0.770	0.056	-13.837	8.476	-0.897 to -0.643	<0.001*	
Iron Deficiency Anemia Females	FORT	3.00	0.68	1.030	0.148	6.972	32.064	0.729 to 1.331	<.001*	(Yoo, Maeng et al. 2009)
	FORD	0.92	0.40	-0.570	0.088	-6.512	31.476	-0.748 to -0.392	<.001*	
Sickle Cell Disease	FORT	3.42	0.94	1.4500	0.173	8.385	42.115	1.101 to 1.799	<.001*	(Gizi, Papassotiriou et al. 2011)
	FORD	1.22	0.20	-0.270	0.062	-4.373	12.577	-0.404 to -0.136	<.001*	
Controls (Combined)	FORT	1.97	0.64	0.000	0.180	0.000	73.283	-0.358 to 0.358	>.999	N/A
	FORD	1.42	1.27	-0.070	0.132	-0.530	98.433	-0.332 to 0.192	0.598	
Recreationally Active WomenOC	FORT	3.22	0.49	1.250	0.194	6.427	10.413	0.819 to 1.681	<.001*	(Quinn, Cox et al. 2020)
	FORD	1.30	0.07	-0.190	0.059	-3.247	9.911	-0.321 to -0.059	0.009	
Recreationally Active Males	FORT	1.74	0.24	-.230	0.132	-1.743	11.167	-0.060 to .520	0.109	
	FORD	1.37	0.07	-0.120	0.060	-1.992	10.425	-0.253 to -0.013	0.073	
Well Trained Male Participants	FORT	1.88	0.32	-0.090	0.128	-0.704	17.419	-0.179 to 0.359	0.491	(Lewis, Newell et al. 2016)
	FORD	1.56	0.15	0.070	0.068	1.022	15.157	0.076 to 0.216	0.323	
Elite UK Endurance Athletes	FORT	1.66	0.36	-0.310	0.128	-2.417	19.264	-0.042 to -0.578	0.026	
	FORD	1.76	0.17	0.270	0.069	3.922	16.098	0.124 to 0.416	0.001#	
Elite Endurance Track Males	**FORT**	**1.66**	**0.22**	**-0.310**	**0.104**	**-2.978**	**12.589**	**-0.084 to 0.536**	**0.011**	N/A
	FORD	**1.50**	**0.16**	**0.010**	**0.066**	**0.151**	**14.969**	**0.152 to 0.132**	**0.882**	
Elite Endurance Track Females	**FORT**	**1.97**	**0.25**	**Reference Group**						
	FORD	**1.49**	**0.15**							

Bonferroni correction for multiple comparisons indicates significance at p<0.003. ***** Indicates significantly higher FORT and lower FORD values relative to male and female elite endurance track athletes. # Indicates significantly higher FORD value relative to male and female elite endurance track athletes.

An analysis of individual sample points from elite endurance track athletes showed that out of the 744 individual FORT samples, only two (0.27%) (one from a male athlete (3.55 mmol) and one from a female athlete (4.56 mmol) exceeded the weighted mean FORT value (3.06 mmol) for the reported disease populations listed in Table 2. In contrast, 77 out of the 734 individual FORD values (54 from male and 23 from female athletes) from the EETA sample (10.49%) were below the weighted mean FORD value (0.94 mmol) for the reported disease populations listed in Table 2. However, alterations in redox balance were not sustained in most cases. Both athlete's FORT level returned below the clinical (i.e., weighted mean) value at the very next sample point and in all but 14 occasions, each athlete's FORD value retuned above clinical (i.e., weighted mean) value at the very next sample point. Manufacture described FORT classification for "strong oxidative stress" of >3.08mmol almost exactly coincided with the weighted mean value calculated from the reported clinical sample of 3.06mmol, and Manufacture described FORD classification of "reduced antioxidant capacity" of <1.07mmol corresponded well with the weighted mean value calculated from the reported clinical sample of 0.94mmol (Kamhieh-Milz & Salama, 2014).

Oxidative stress is elevated during pre-season training of elite endurance track athletes

Combined means and standard deviation (SD) for male and female athletes for FORT, FORD and OSI for each phase are listed in Table 3.

Table 3: Redox values across the competitive year by periodization phase. Data are combined means ± SD for male and female athletes.

	Preparatory		Competitive		Transition
FORT	1.76±0.29		1.63±0.26		1.71 0.36
FORD	1.45±0.27		1.49±0.26		1.55±0.18
OSI	1.37±0.50		1.25±0.46		1.13±0.29
n	93		76		20
	General Prep	**Specific Prep**	**Pre-Competition**	**Competition**	**Transition**
FORT	1.74±0.28	1.77±0.30	1.70±0.26	1.69±0.28	1.71±0.36
FORD	1.43±0.28	1.47±0.26	1.48±0.26	1.52±0.25	1.55±0.18
OSI	1.42±0.61	1.33±0.40	1.29±0.52	1.18±0.33	1.13±0.29
n	40	53	52	24	20

Monthly alterations in FORT, FORD and OSI across the competitive year are shown in Figure 2 and OSI across the competitive year by general periodization phase, sub-category and month are shown in Figure 3. There was no significant difference in FORT values between general training phases, sub-categories, or months. There was a significant difference in FORD values between general training phases [$F(2, 173.04) = 4.233$, $p=0.016$], sub-category [$F(4, 167.22) = 2.657$, $p=0.035$] and month [$F(11, 157.41) = 3.235$, $p<0.001$], with post-hoc analysis revealing significantly higher FORD values in the Preparatory phase relative to the Transition phase ($p=0.017$), the General Preparation phase relative to the Transition phase ($p=0.028$), and in December relative to January ($p=0.013$), June ($p=0.043$), October ($p=0.004$) and November ($p=0.040$). Likewise, there was a significant difference in OSI values between general training phases [$F(2, 172.95) = 6.051$, $p=0.003$], sub-category [$F(4, 166.92) = 3.946$, $p=0.004$] and

month [F (11, 156.75) = 4.240, p<0.001], with post-hoc analysis revealing significantly higher OSI values in the Preparatory phase relative to the Transition phase (p=0.005), the General Preparation phase relative to the Transition phase (p=0.007), and in December relative to January (p=0.011), May (=0.021), June (p=0.004), July (0.024), August (0.005), September (p=0.012), October (p<0.001) and November (p=0.010).

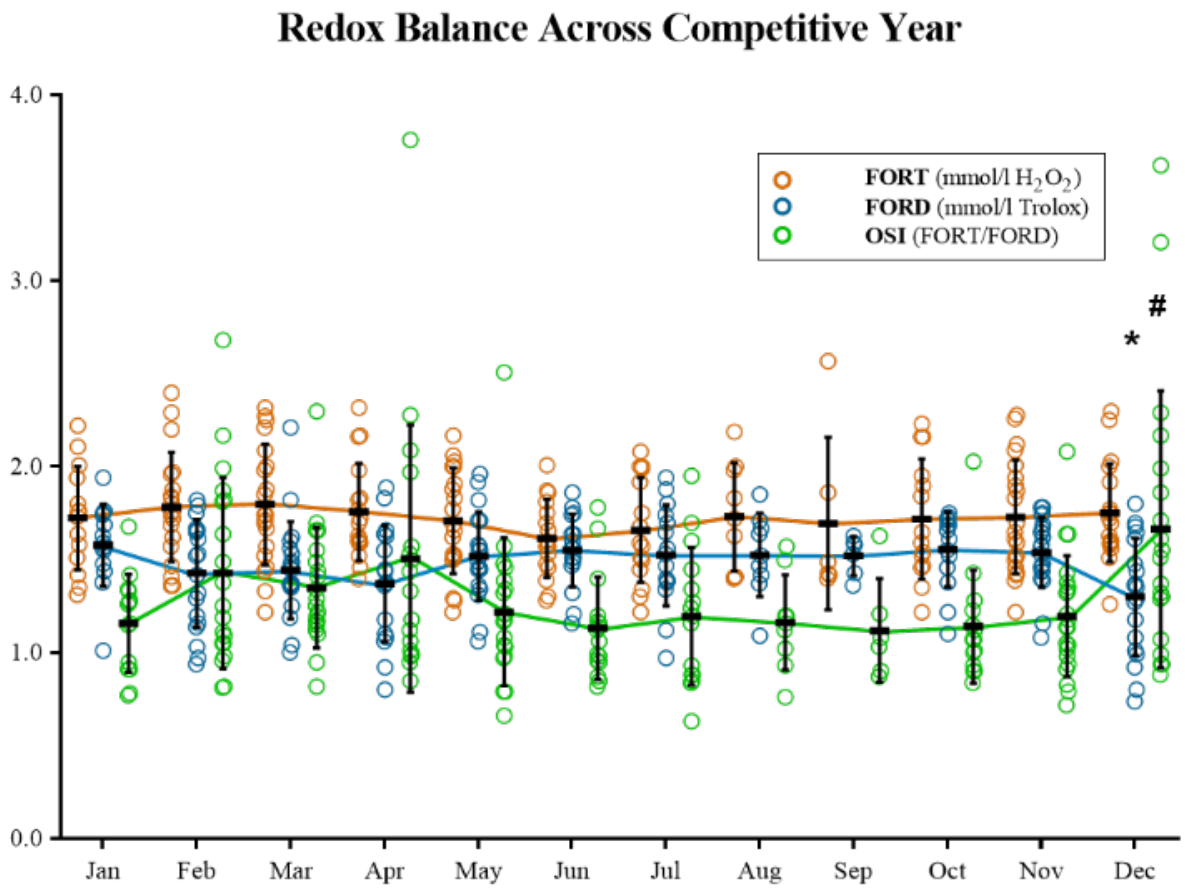

Figure 2. Alterations in redox biomarkers across the competitive year. Open circles represent mean values per month for each individual and error bars are mean and SD. * Indicates FORD significantly lower than January, June, October and November, p<0.044 # indicates OSI significantly higher than January, May, June, July, August, September, October and November, p<0.022.

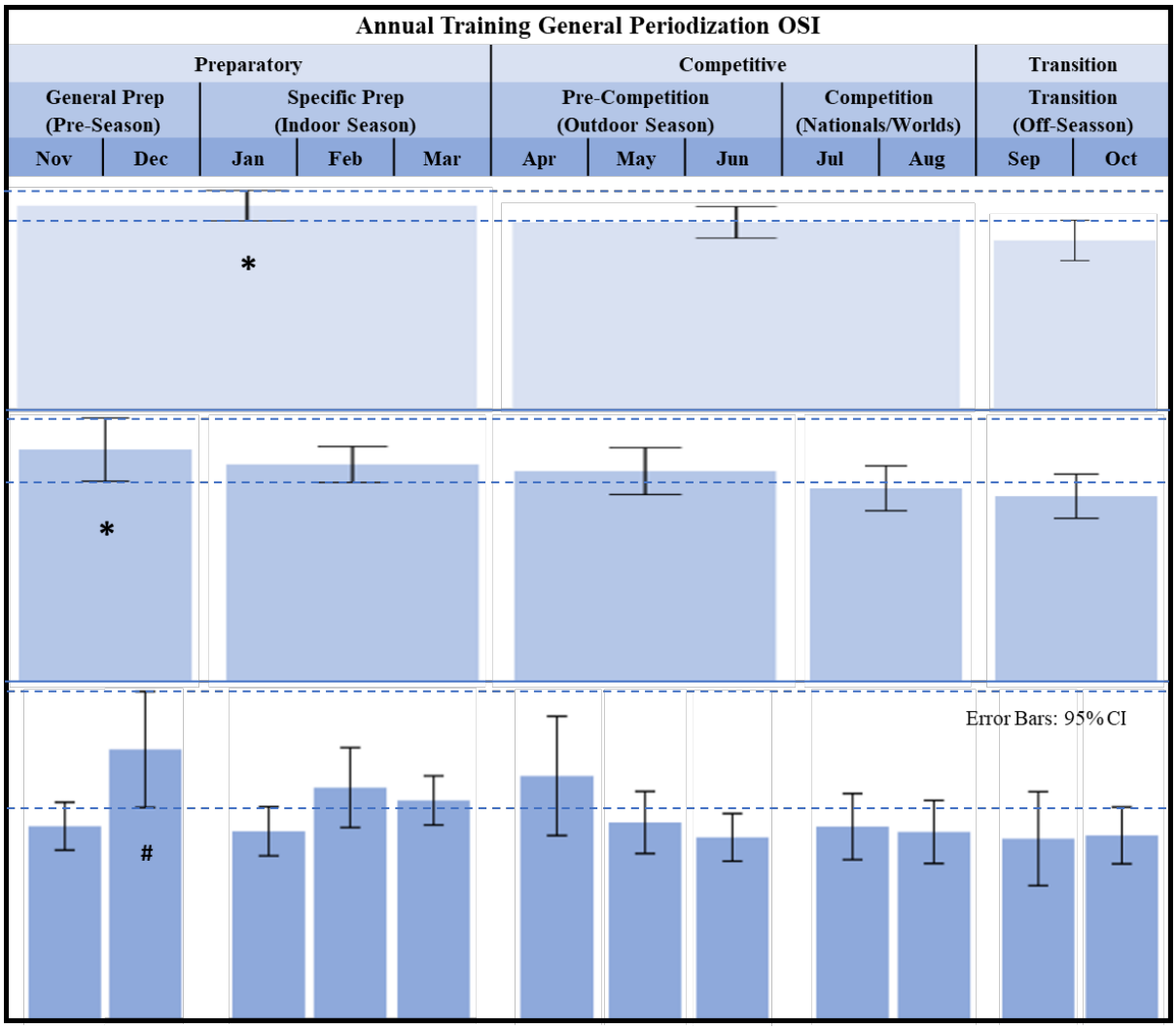

Figure 3. Oxidative stress balance (OSI) across the competitive season by general periodization phase and month. Numerical values are listed in Table 3. * Indicates significantly higher than transition phase and # indicates significantly higher than every other month except February, March and April, $p<0.012$.

The impact of sex across the competitive year by periodization sub-category was also evaluated for FORD, FORT and OSI. There was a significant main effect of sex for FORT and OSI, but not FORD, with no significant interaction effects between period and sex for either FORT, FORD or OSI. Post Hoc analysis revealed a significant difference between Male and Female FORT values for every period across the competitive year (General Preparation (EMM±SE: 2.078±0.084 vs 1.628±0.057, $p<0.001$), Specific Preparation (2.078±0.079 vs 1.649±0.054, $p<0.001$), Pre-Competition (1.933±0.080 vs 1.616±0.055, $p=0.003$),

Competition (1.954±0.099 vs 1.579±0.062, p=0.002) and Transition (1.929±0.087 vs 1.528±0.072, p<0.001) and OSI values for Specific Preparation (1.630±0.142 vs 1.327±0.090, p=0.005) and Pre-Competition phases (1.636±0.129 vs 1.120±0.085, p=0.001). Mean FORT and OSI values across the competitive year by sex are shown in Figure 4 and Figure 5, respectively.

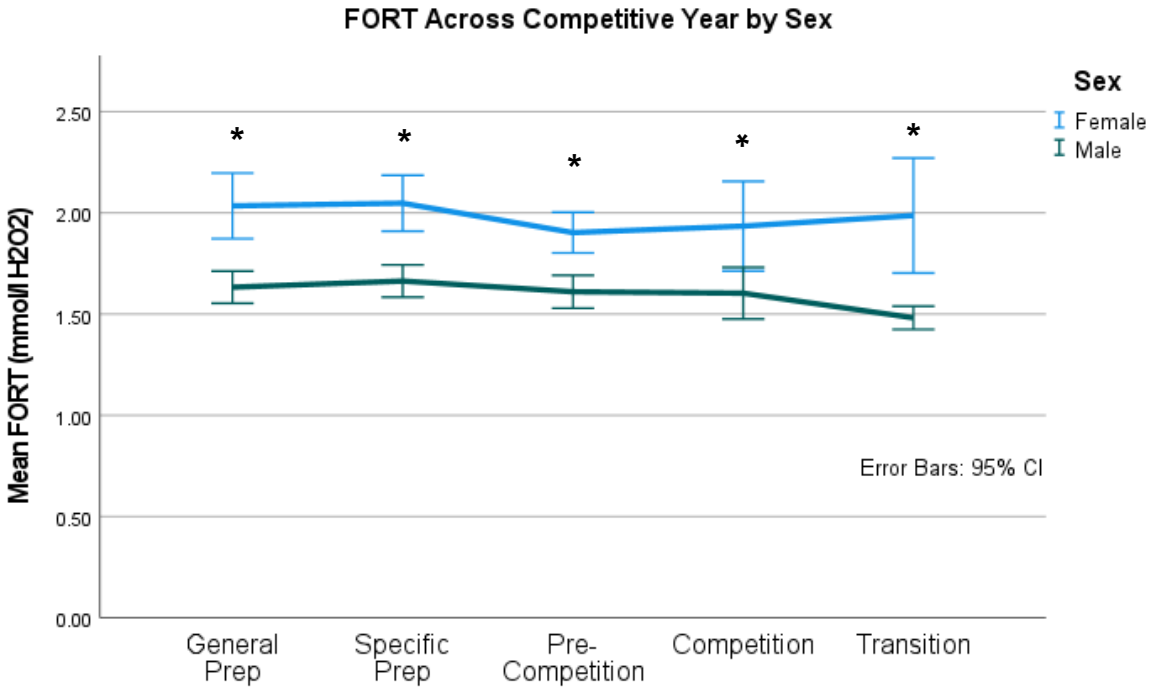

Figure 4. Mean FORT across the competitive year by sub-category periodization phase and sex. * Indicates Females significantly higher than Males, p<0.004.

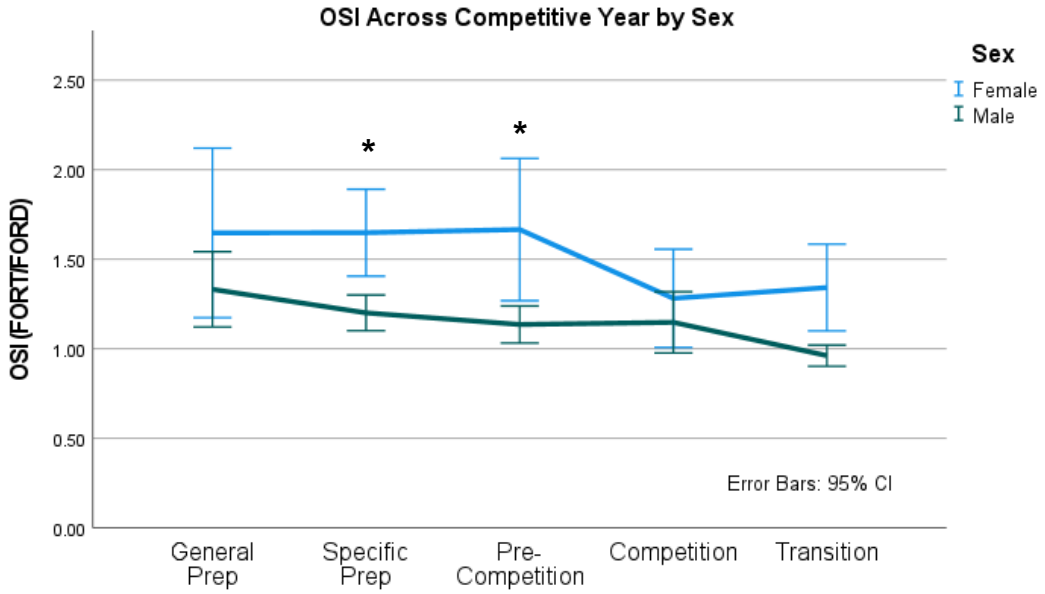

Figure 5. Mean OSI across the competitive year by sub-category periodization phase and sex. * Indicates Females significantly higher than Males, $p<0.006$.

Moderate altitude exposure alone did not impact PoC biomarkers of oxidative stress in elite endurance track athletes

Redox values from 8 elite endurance track athletes (5 male and 3 female) sampled during an altitude training camp (base elevation of approximately 7000 feet) in March and April of 2019 were used for this analysis. Statistical analysis revealed no main effect of timepoint for FORT, FORD or OSI [$F(4, 27.34) = 0.618$, $p=0.654$; $F(4, 26.88) = 1.879$, $p=0.143$; $F(4, 26.41) = 0.671$, $p=0.618$, respectively]. Alterations in redox balance at altitude are shown in Figure 6.

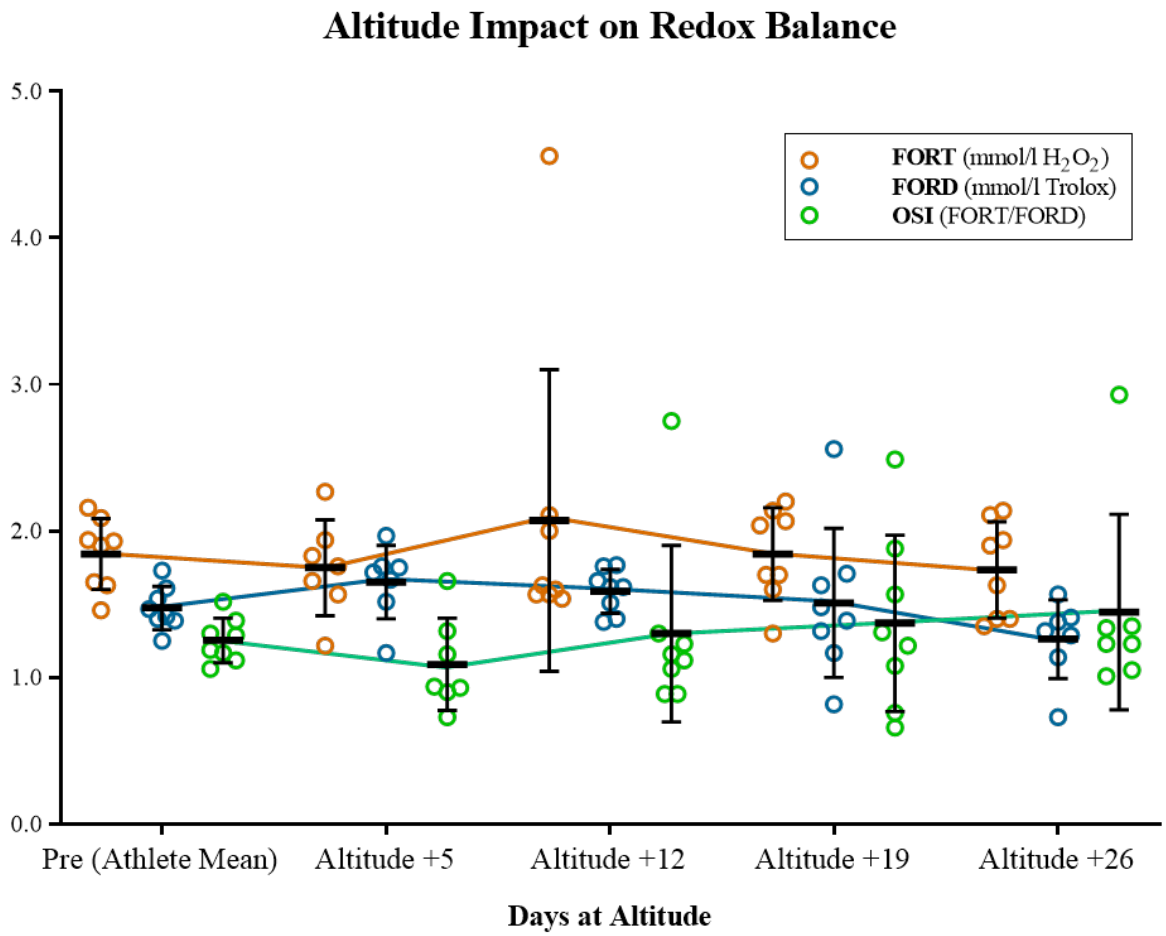

Figure 6. Alterations in redox balance at altitude. Open circles are individual datapoints and error bars are mean and SD. *Sea level datapoint was calculated from individual mean FORT, FORD and OSI values for each athlete. No significant differences between timepoints.

Redox values for each athlete at altitude are listed in Table 6 and figures showing individual alterations in FORT, FORD and OSI throughout the duration of the altitude training camp are shown in Figure 13, Figure 14 and Figure 15 in the Supplementary Data Section.

*

COVID-19 mRNA vaccination plus altitude transiently increased oxidative stress and inflammation in some but not all elite endurance track athletes

Redox values from 9 elite endurance track athletes (6 male and 3 female) at an altitude training camp were sampled before, during (between vaccine shots 1 and 2) and after COVID-19 mRNA vaccination (SPIKEVAX™, Moderna Co., Cambridge, MA, USA) in March and April of 2021. The date the 1st dose was administered was considered day zero and the 2nd dose was administered on day 28±3. Only two hsCRP samples were collected for the timepoint D1+19±1 and only three for timepoint D1+26±1 (no overlapping samples), therefore D1+19±1 and D1+26±1 were combined into a single D1+25±5 timepoint for hsCRP. Statistical analysis revealed no main effect of timepoint for FORT, however there was a main effect of timepoint for FORD and OSI and hsCRP [$F (13, 88.567) = 5.110$, $p<0.001$; $F (13, 85.059) = 2.614$, $p=0.004$; $F (12, 71.453) = 2.007$, $p=0.036$, respectively]. Post Hoc analysis revealed a significant difference between vaccination period timepoints for FORD, OSI and hsCRP, which are shown in Table 6, Table 7 and Table 8, respectively. In summary, the greatest alteration in redox balance and inflammation were seen in the first 6 days after vaccination plus altitude exposure (reduction in FORD and increases in OSI and hsCRP), with some reduction in antioxidative capacity (i.e., FORD) continuing until 6 days after the administration of the second vaccine dose and return to sea level. It is of note that vaccination plus altitude exposure was associated with a sharp increase in hsCRP between 1- and 6-days post vaccination in 5 athletes but showed no effect on the 4 other athletes. There were no significant changes in redox balance or inflammation after administration of the second vaccine dose and return to sea level.

Table 4: Pairwise comparison of vaccination timepoints for FORD. Timepoints are days before or after vaccine dose 1 (i.e., D1) or vaccine dose 2 (i.e., D2). Days are ±SD).

Timepoints (Dose±Days±SD)		Mean Difference	SE	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound	df	Significance
1st Dose +1±0	**D1-63±3**	-0.650	0.155	-0.958	-0.342	90.505	<0.001
	D1-56±3	-0.448	0.155	-0.756	-0.140	90.505	0.005
	D1-49±3	-0.313	0.150	-0.612	-0.015	89.878	0.040
	D1-43±4	-0.525	0.155	-0.833	-0.217	90.495	0.001
	D1-36±4	-0.471	0.150	-0.770	-0.172	89.878	0.002
	D1-25±8	-0.362	0.155	-0.670	-0.054	90.491	0.022
	D1-6±3	-0.326	0.150	-0.625	-0.028	89.615	0.033
	D2+19±6	-0.319	0.146	-0.610	-0.028	89.044	0.032
1st Dose +6±1	**D1-63±3**	-0.641	0.140	-0.919	-0.363	88.677	<0.001
	D1-56±3	-0.439	0.140	-0.717	-0.161	88.677	0.002
	D1-49±3	-0.304	0.135	-0.572	-0.037	88.185	0.026
	D1-43±4	-0.516	0.140	-0.794	-0.238	88.658	<0.001
	D1-36±4	-0.462	0.135	-0.730	-0.194	88.185	0.001
	D1-25±8	-0.353	0.140	-0.631	-0.075	88.664	0.013
	D1-6±1	-0.317	0.135	-0.585	-0.050	88.023	0.021
	D2+19±6	-0.310	0.130	-0.569	-0.051	87.568	0.020
1st Dose +12±1	**D1-63±3**	-0.599	0.140	-0.876	-0.321	88.677	<0.001
	D1-56±3	-0.397	0.140	-0.675	-0.119	88.677	0.006
	D1-43±4	-0.474	0.140	-0.752	-0.196	88.658	0.001
	D1-36±4	-0.420	0.135	-0.687	-0.152	88.185	0.002
	D1-25±8	-0.311	0.140	-0.589	-0.033	88.664	0.029
	D1-6±3	-0.275	0.135	-0.542	-0.007	88.023	0.044
	D2+19±6	-0.268	0.130	-0.527	-0.008	87.568	0.043
1st Dose +19±1	**D1-63±3**	-0.642	0.140	-0.920	-0.364	88.677	<0.001
	D1-56±3	-0.440	0.140	-0.718	-0.163	88.677	0.002
	D1-49±3	-0.306	0.135	-0.573	-0.038	88.185	0.026
	D1-43±4	-0.517	0.140	-0.795	-0.240	88.658	<0.001
	D1-36±4	-0.463	0.135	-0.731	-0.195	88.185	0.001
	D1-25±8	-0.354	0.140	-0.632	-0.077	88.664	0.013
	D1-6±3	-0.318	0.135	-0.586	-0.051	88.023	0.020
	D2+19±6	-0.311	0.130	-0.570	-0.052	87.568	0.019
1st Dose +26±1	**D1-63±3**	-0.751	0.155	-1.059	-0.442	90.527	<0.001
	D1-56±3	-0.549	0.155	-0.857	-0.241	90.527	0.001
	D1-49±3	-0.414	0.150	-0.713	-0.116	89.891	0.007
	D1-43±4	-0.626	0.155	-0.933	-0.319	89.485	<0.001
	D1-36±4	-0.572	0.150	-0.870	-0.273	89.891	<0.001
	D1-25±8	-0.463	0.155	-0.771	-0.155	90.514	0.004
	D1-6±3	-0.427	0.150	-0.726	-0.128	89.639	0.006
	D2+19±6	-0.420	0.146	-0.711	-0.129	89.059	0.005
2nd Dose +6±2	**D1-63±3**	-0.527	0.140	-0.805	-0.250	88.677	<0.001
	D1-56±3	-0.326	0.140	-0.604	-0.048	88.677	0.022
	D1-43±4	-0.403	0.140	-0.681	-0.125	88.658	0.005
	D1-36±4	-0.349	0.135	-0.616	-0.081	88.185	0.011
2nd Dose +19±6	**D1-63±3**	-0.331	0.140	-0.609	-0.053	88.677	0.020
	D1+1±0	0.319	0.146	0.028	0.610	89.044	0.032
	D1+6±1	0.310	0.130	0.051	0.569	87.568	0.020
	D1+12±1	0.268	0.130	0.008	0.527	87.568	0.043
	D1+19±1	0.311	0.130	0.052	0.570	87.568	0.019
	D1+26±1	0.420	0.146	0.129	0.711	89.059	0.005

Note: Only significant differences are shown for clarity. P<0.05

Table 5: Pairwise comparison of vaccination timepoints for OSI. Timepoints are days before or after vaccine dose 1 (i.e., D1) or vaccine dose 2 (i.e., D2). Days are ±SD).

Timepoints (Dose±Days±SD)		Mean Difference	SE	95% Confidence Interval		df	Significance
				Lower Bound	Upper Bound		
1st Dose +1	**-63±3**	0.532	0.234	0.068	0.997	84.683	0.025
1st Dose +6	**-63±3**	0.848	0.210	0.432	1.265	83.933	<0.001
	-56±3	0.676	0.210	0.259	1.092	83.933	0.002
	-49±3	0.44	0.202	0.039	0.841	83.771	0.032
	-43±4	0.611	0.220	0.174	1.048	84.075	0.007
	-36±4	0.652	0.202	0.251	1.053	83.771	0.002
	-25±8	0.658	0.210	0.241	1.074	83.933	0.002
	-6±3	0.575	0.210	0.158	0.991	83.933	0.007
	+12±1	0.397	0.195	0.009	0.785	83.425	0.045
	+6±2	0.402	0.201	0.001	0.802	83.577	0.049
	+19±6	0.627	0.195	0.239	1.015	83.425	0.002
1st Dose +12	**-63±3**	0.451	0.210	0.034	0.868	83.933	0.034
	+6±1	-0.397	0.195	-0.785	-0.009	83.425	0.045
1st Dose +19	**-63±3**	0.519	0.210	0.103	0.936	83.933	0.015
1st Dose +26	**-63±3**	0.795	0.234	0.330	1.259	84.715	0.001
	-56±3	0.622	0.234	0.157	1.087	84.715	0.009
	-43±4	0.557	0.242	0.076	1.038	84.544	0.024
	-36±4	0.598	0.226	0.149	1.048	84.492	0.010
	-25±8	0.604	0.234	0.139	1.068	84.715	0.011
	+6±1	0.521	0.234	0.056	0.986	84.715	0.028
	+19±6	0.573	0.220	0.137	1.010	83.966	0.011
2nd Dose +6	**-63±3**	0.447	0.216	0.017	0.876	84.164	0.042
	+6±1	-0.402	0.201	-0.802	-0.001	83.577	0.049
2nd Dose +19	**+6±1**	-0.627	0.195	-1.015	-0.239	83.425	0.002
	+26±1	-0.573	0.220	-1.010	-0.137	83.966	0.011

Note: Only significant differences are shown for clarity.

Table 6: Pairwise comparison of vaccination timepoints for hsCRP. Timepoints are days before or after vaccine dose 1 (i.e., D1) or vaccine dose 2 (i.e., D2). Days are ±SD).

Timepoints (Dose±Days±SD)		Mean Difference	SE	95% Confidence Interval		df	Significance
				Lower Bound	Upper Bound		
1st Dose +1±0	D1-63±3	3.064	1.120	0.830	5.297	71.882	0.008
	D1-56±3	3.885	1.120	1.652	6.118	71.882	0.001
	D1-43±4	3.682	1.119	1.450	5.913	71.827	0.002
	D1-36±4	3.907	1.083	1.749	6.066	71.734	0.001
	D1-25±8	3.633	1.120	1.400	5.866	71.869	0.002
	D1-6±3	3.205	1.080	1.052	5.358	71.499	0.004
	D1+6±1	2.153	1.050	0.059	4.246	71.381	0.044
	D1+12±1	3.311	1.446	0.428	6.193	72.314	0.025
	D1+25±5	2.962	1.219	0.532	5.391	71.687	0.018
	D2+6±2	2.157	1.050	0.064	4.251	71.381	0.044
	D2+19±6	3.905	1.050	1.811	5.998	71.381	<0.001
1st Dose +6±1	D1+1±0	-2.153	1.050	-4.246	-0.059	71.381	0.044
1st Dose +12±1	D1+1±0	-3.311	1.446	-6.193	-0.428	72.314	0.025
1st Dose +25±5	D1+1±0	-2.962	1.219	-5.391	-0.532	71.687	0.018
2nd Dose +6±2	D1+1±0	-2.157	1.050	-4.251	-0.064	71.381	0.044
2nd Dose +19±6	D1+1±0	-3.905	1.050	-5.998	-1.811	71.381	<0.001

Individual alterations in FORT, FORD, OSI and hsCRP throughout the vaccination plus altitude period are represented in Figure 7, Figure 8, Figure 9 and Figure 10, with an overlay of altitude alone data from a 2019 altitude training camp at the same location and included four of the same athletes. Combined mean and standard deviation for FORT, FORD and OSI from all athletes throughout the duration of the vaccination plus altitude period are shown in Figure 16 and redox values for each athlete throughout the vaccination period are listed in Table 7 in the Supplementary Data Section.

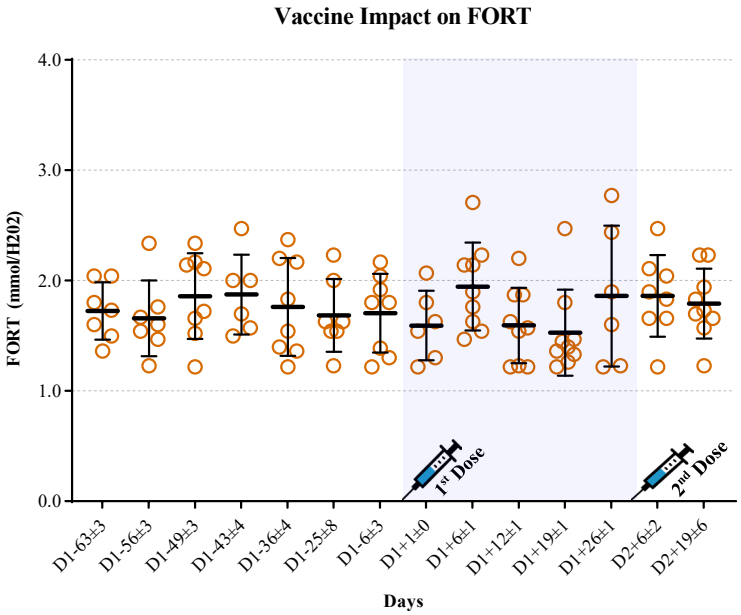

Figure 7. Vaccine impact on FORT. Open circles are individual sample points for each athlete. Shaded area depicts time between vaccination doses. Days are mean ±SD. No significant difference in FORT values throughout vaccination period.

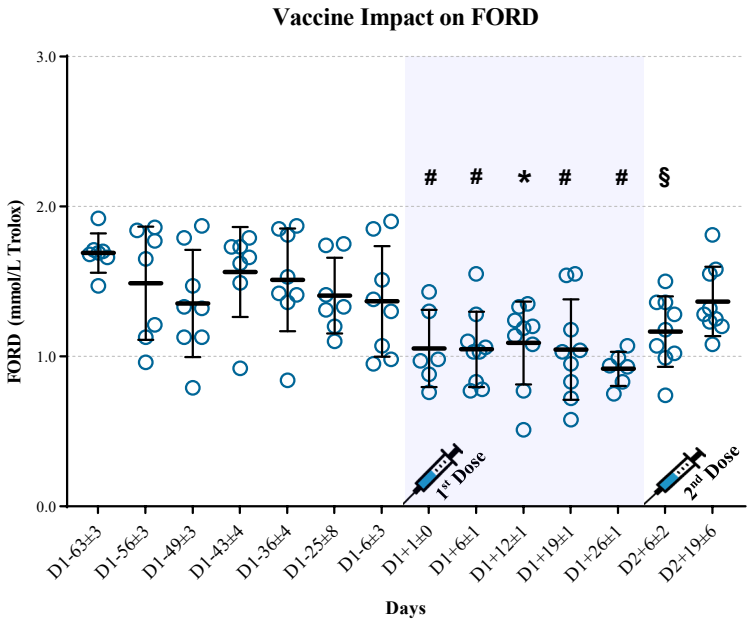

Figure 8. Vaccine impact on FORD. Open circles are individual sample points for each athlete. Shaded area depicts time between vaccine doses. Days are mean ±SD. # Indicates significantly lower FORD value relative to all pre-vaccine timepoints, $p<0.044$. * Indicates significantly lower FORD value than all pre-vaccine timepoints except D1-49±3 and D1-6±3 days, $p<0.031$. § Indicates significantly lower FORD value than all pre-vaccine timepoints except D1-49±3, D1-25±8 and D1-6±3 days, $p<0.027$.

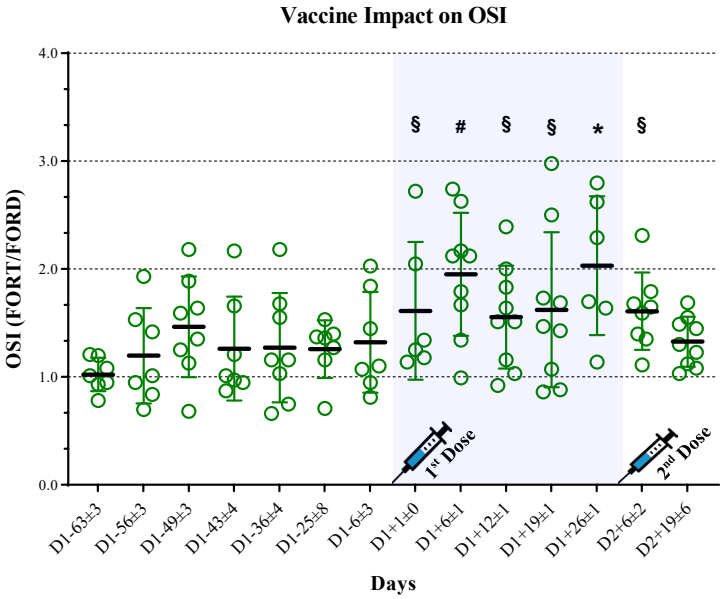

Figure 9. Vaccine impact on OSI. Open circles are individual sample points for each athlete. Shaded area depicts time between vaccine doses. Days are mean ±SD. # indicates significantly higher OSI value relative to all pre-vaccine timepoints, p<.0.033. * Indicates significantly higher OSI value than all pre-vaccine timepoints except D1-49±3 and D1-6±3 days, p<0.029. § Indicates significantly higher OSI value than D1-63±3, p<0.043.

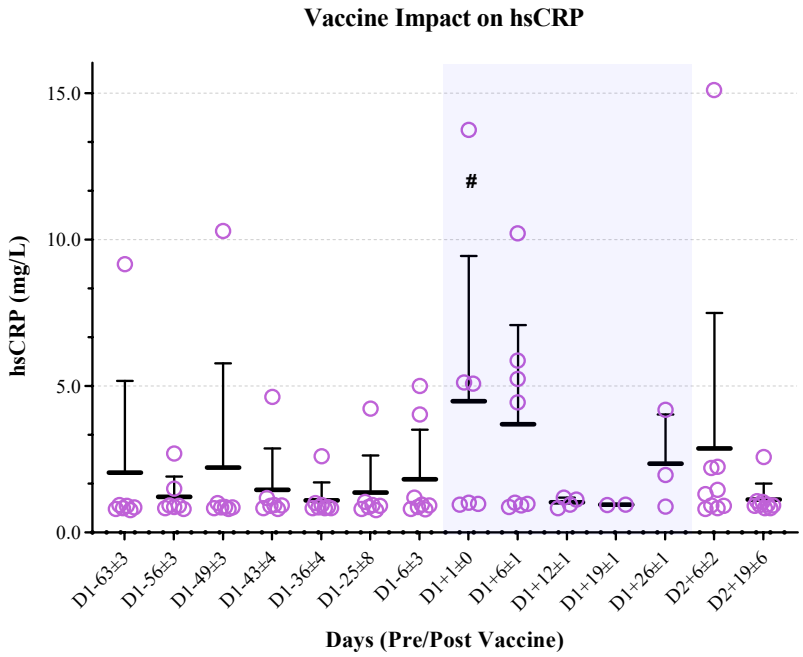

Figure 10. Vaccine impact on hsCRP. Open circles are individual sample points for each athlete. Shaded area depicts time between vaccine doses. Days are mean ±SD. # indicates significantly higher hsCRP value relative to all other timepoints except D1-49±3, p<.0.045.

Chapter 4

Discussion

Comparison of PoC biomarkers of oxidative stress across independent sample groups

Extensive endurance exercise is known to alter redox homeostasis (Lewis, 2015), however the severity of the exercise induced oxidative stress relative to levels associated disease is not well understood. The aim of this study was to compare PoC biomarkers of oxidative stress in elite endurance track athletes with published data for disease states and sedentary controls. The primary finding was that mean oxidative stress levels in elite endurance track athletes are lower than reported disease states but not significantly different than healthy controls or other characterized athletic populations (except for FORD values of Elite UK Endurance Athletes, which were significantly higher). The secondary finding was that although levels were not sustained, elite endurance athletes did experience transient elevations of oxidative stress into ranges associated with disease states (i.e., beyond weighted mean values associated with the reported clinical populations and manufacturer reported normal ranges), particularly in terms of reduction of anti-oxidative capacity (i.e., FORD). However, when alterations in athlete redox balance exceeded clinically relevant levels, the response was predominantly dynamic, quickly returning to within normal ranges. These results indicate that though there are transitory excursions into levels associated with disease, the extensive training of elite endurance track athletes did not result in sustained redox alteration comparable with reported clinical (i.e., disease) populations.

This was the first study to compare PoC biomarkers of redox balance in athletes with disease states and heathy controls. This work showed that individuals with clinical disorders,

specifically including diabetes, obesity, iron deficiency anemia and sickle cell disease exhibit a disruption of redox balance, including both an increase in pro-oxidant status (i.e., FORT) and decrease in anti-oxidative capacity (i.e., FORD) (M. A. Gaman et al., 2020). Conspicuously, active women using oral contraceptives (WomanOC) showed an increase in pro-oxidant status (i.e., FORT) commensurate with reported clinical populations (Quinn et al., 2020). Curiously, the sample of Type II diabetic women reported lower mean FORT values than healthy, active women using oral contraceptives (i.e., WomanOC), yet this could be explained by the age difference in age of the samples (i.e., WomenOC: 24±5 years old, Type II Diabetic Women: 55.1±11.8 years old) which likely indicates that most of the diabetic women studied were beyond child bearing age and thus not using oral contraceptives (Pavlatou et al., 2009). Elite UK endurance athletes were the only group that had a more favorable redox balance than the elite endurance track athletes, with a significantly higher anti-oxidative capacity (i.e., FORD) (Lewis, Newell, et al., 2016). Noticeably, this group of elite UK athletes were reported to be training out of a UK National Training Center, which could explain the athletes more favorable redox balance as studies of athletes training at UK National Training Centers have highlighted both the monitoring the redox balance of their elite athletes (Lewis et al., 2020) as well as the use of anti-oxidative nutritional interventions (Lewis et al., 2018), which could account for the increase in anti-oxidative capacity.

There are a number of studies reporting oxidative balance using PoC biomarkers that could not be included in the statistical comparison shown in Figure 1 due to lack of complete summary data (i.e., means, SD and sample size); however, qualitatively they support the conclusion that despite extensive training, elite endurance track athletes maintained a more favorable redox balance relative to reported clinical states. In a study by Gaman et al., PoC

biomarkers were assessed in patients with chronic lymphocytic leukemia (CLL) and though means and standard deviation were not documented, the reported range of FORT was 2.5-3.8 mmol/l and the reported range of FORD was 0.39-0.96 mmol/l, showing a marked disruption of redox balance in all 84 patients involved in the study (A. M. Gaman, Buga, Gaman, & Popa-Wagner, 2014). These authors also assessed the use of antioxidants as an adjuvant to chemotherapy and although they did not find a significant change in redox status with the intervention, they did report greater tolerance to chemotherapy and decreased infections complications in the intervention group relative to controls (i.e., CLL patients undergoing chemotherapy without the antioxidant intervention) (A. M. Gaman et al., 2014). A study of patients with coronary atherosclerosis (CA) by Shramko et al. showed elevated mean FORT values (2.27 mmol/l vs 1.94 mmol/l) and decreased FORD levels (0.59 mmol/l vs 1.08 mmol/l) relative to healthy controls, with no standard deviations reported (Shramko et al., 2018). Neurological disorders such as Alzheimer's and Parkinson's disease have shown oxdative stress related pathologies (Talebi et al., 2022), though a study by Tarani et al. found no difference in PoC redox balance in down syndrome children relative to healthy controls (Tarani et al., 2020), however the pathogenic mechanisms of Down syndrome are still largely unknown (Aivazidis et al., 2017). The finding that females using oral contraceptives had significantly elevated FORT levels relative to athletes and healthy controls was substantiated by two additional studies that could not be included in the statistical comparison. Cauci et al. evaluated the effect of oral contraceptives on the redox balance of 144 female athletes, including 27 elite athletes, and they reported that FORT was almost two-fold higher in female athletes using oral contraceptives relative to female athletes not using oral contraceptives (means and SD not reported) (Cauci et al., 2016). Quinn et al. also evaluated the effect of oral contraceptives on

the redox balance of active females and reported WomenOC (active women using oral contraceptives) showed a significant increase in FORT, OSI, MDA and CRP relative to women who were not using oral contraceptives, though no summary data was reported (Quinn et al., 2021). Three additional PoC studies of athletes were found that did not include summary data for analysis but supported the comparative conclusion that athletes maintained a favorable redox balance, including studies on American football players (McKay et al., 2021), Olympic rowers (Lewis et al., 2020), and elite runners and triathletes (Lewis, Towey, et al., 2016).

In addition to group mean values, individual alterations of redox balance in elite endurance track athletes were evaluated to determine if there were any excursions into ranges associated with reported clinical conditions. The second finding of the current study was that although aberrant levels were not sustained, elite endurance athletes did experience transient elevations of oxidative stress into ranges associated with disease states (i.e., beyond weighted mean levels associated with the reported clinical populations and manufacturer reported normal ranges), particularly in terms of reduction of anti-oxidative capacity (i.e., FORD). Even though no comparison of kinetics could be made with clinical populations because no study has reported repeated measures of PoC biomarkers in disease populations other than pre and post intervention samples, the striking observation with elite endurance track athletes was that alterations in redox balance were very dynamic. Excursions into oxidative ranges associated with clinical states recovered quickly on most occasions (69.8%), with 30/43 excursions returning to within sub-clinical ranges at the very next sample point, regardless of the magnitude of the excursion. There were seven occasions where the depression of anti-oxidative capacity was sustained for an extended period (i.e., a matter of weeks or months), which is an

indication that additional rest/recovery and/or nutritional intervention may have been of value during this period, though because no performance data was captured, further study is needed.

The redox basis of exercise physiology has been well characterized (Margaritelis et al., 2020b), with the relative stress (i.e., the magnitude of stress relative to recent and historical individual experience) of an acute exercise challenge governing the response. If the relative stress of an acute exercise bout is too low, then adaptive shift in redox balance is limited (Margaritelis et al., 2020b). If the relative stress of acute exercise challenge is higher, but well within athlete resilience, then there appears to be an immediate adaptive shift to a more favorable redox balance (Lewis, Towey, et al., 2016). If the relative stress of an acute exercise bout is higher still, in the upper range of athlete resilience, then there appears to be an immediate adaptive shift to a less favorable redox balance (Quinn et al., 2020). And if relative stress is excessive and/or sustained, such as with over-trained athletes, then the ability to dynamically adapt can become altogether compromised (Tanskanen et al., 2010). Therefore, particularly in athletes, it appears that the dynamic ability to adapt to stressors is paramount, not the absolute value of transient alterations in redox balance. In fact, because dynamic redox signaling is integral to the intended adaptive process, the theory of *adaptive homeostasis* has been proposed which postulates that transient exercise induced shifts in redox balance are what drive the adaptive process, with greater transient shifts potentially providing a stronger response as long as relative stress is withing the adaptive capacity of the athlete (Davies, 2018). The theory of *adaptive homeostasis* aligns with the governing theory of *exercise hormesis* by Radak et al., which highlights the importance biological balance, transience of redox alteration and dynamic modulation of the adaptive response via expression of regulatory factors (Radak et al., 2017; Radak et al., 2020).

There are several limitations to this study. First, this research compares oxidative stress values of our athlete sample to those in the literature to gauge clinical significance, and although sampling and analysis should be uniform as there is only one company producing these PoC biomarker analysis kits, there is no way to verify the accuracy of the reported values. Second, all but one study of disease states reported significant disruption in redox balance, but these studies are limited to clinical areas where oxidative stress related pathologies are known. Not every clinical disorder will show disruptions in oxidative balance, however for the ones found to be redox related, due to the ease of application, PoC biomarkers may be of particular value as patients could actively track their management of the disease at home if the technology was scaled. Third, there is a venerable body of redox research on both athletes and clinical disorders that used biomarkers other than PoC biomarkers and therefore no direct comparison could be performed.

In conclusion, the main finding was that mean oxidative stress levels in elite endurance track athletes are lower than reported disease states but not significantly different than healthy controls or most other characterized athletic populations. Elite endurance athletes did experience elevations of oxidative stress into clinically relevant ranges, particularly in terms of reduction of anti-oxidative capacity (i.e., FORD), however these excursions were transient, highlighting biological resiliency and adaptive nature of these athletes. These results indicate that though there are transitory excursions into levels associated with disease, the extensive training of elite endurance track athletes did not result in sustained redox alteration comparable with reported oxidative stress related clinical conditions. Further study comparing PoC biomarker alterations with markers of adaptation, such as expression of regulatory factors,

markers of mitochondrial biogenesis or specific performance metrics, would continue to develop insight into the optimal dynamics of elite athlete redox balance.

Alterations in redox balance over the course of the competitive year for elite endurance track athletes

Elite athletes have been shown to experience seasonal alterations in redox balance with elevated levels of oxidative stress associated with lower performance, illness and injury, yet the seasonal variations in redox balance of elite endurance track athletes have not been well characterized. The aim of this study was to investigate alterations in the redox balance of elite endurance track athletes over the course of the competitive year using PoC biomarkers of oxidative stress. The primary finding was that oxidative stress balance (i.e., OSI) was elevated during pre-season training of elite endurance track athletes, particularly in December relative to every other month of the year except February, March, and April. This increase in oxidative stress was chiefly a function of decreases in anti-oxidative capacity (i.e., FORD). A secondary finding was that pro-oxidant status (i.e., FORT) was elevated in females relative to males throughout the competitive year, with no differential effect of time-in-season between sexes. These results indicate that particular attention should be paid to redox balance early in the annual training cycle while athletes are re-adapting to higher training loads and that sex-specific redox values or target ranges target ranges should be considered.

This is the first study to report seasonal alterations in redox balance in using PoC biomarkers, however seasonal alterations using various other redox biomarkers have been

reported in elite/professional male American football players (Schippinger et al., 2002), male rugby players (Finaud et al., 2006), male alpine skiers (Schippinger et al., 2009), male karate athletes (Pesic et al., 2012), female water polo players (Varamenti et al., 2013), male soccer players (Becatti et al., 2017; Le Moal et al., 2016; Ponce-Gonzalez et al., 2021; Silva et al., 2014), and competitive (i.e., sub-elite) male runners (Munoz Marin et al., 2018). The unifying factor across all analyses is that seasonal alterations of redox balance occur, though the dynamics of the alterations appear to be sport-specific and tied to periods of intensified training. The primary finding of this study was that oxidative stress was highest during pre-season training (i.e., General Preparation phase), where relative training load is the highest for elite endurance track athletes as they are re-adapting to progressively higher training volumes after a recovery period (i.e., off-season) from the previous season. These findings are in agreement with the work by Munoz Marin et al., which also found the pro-oxidant status (i.e., lipid peroxidation indexes) was highest during pre-season (i.e., General Preparation phase) training relative to 3, 6 and 9 months into the season for middle distance and long-distance competitive runners (Munoz Marin et al., 2018). An earlier work by Schippinger et al. showed a similar pattern of elite athlete adaptation to training when they found pro-oxidant status (i.e., total peroxide levels) were significantly elevated in elite alpine ski racers during early season racing (i.e., November) relative to baseline (i.e., prior to season) values (i.e., July), which then returned back to baseline levels during mid-season racing (i.e., December and January) (Schippinger et al., 2009). That study did not have any sample points between July and November, so redox alterations during the General Preparation phase were not captured. However, work by Finaud et al. evaluating seasonal alterations in professional rugby players reveled lower oxidative stress during the General Preparation (i.e., September) phase relative

to Pre-Competition (i.e., December) and Competition Phase (i.e., April), with the highest pro-oxidant status [i.e., Rmax: maximum rate of conjugated dienes oxidation and PUFA oxidation lag phase (LP)], as well as decreased antioxidative capacity (i.e., TAC) during the Pre-Competition phase (Finaud et al., 2006). Another study by Schippinger et al. compared the Pre-Competition phase (i.e., March) versus the Competition phase (i.e., May, June and July) with male professional American football players and conversely showed higher pro-oxidant status during the later Competition phases [i.e., aAB against oxidized LDL (June only) serum peroxide concentrations (July only)] (Schippinger et al., 2002). Similarly, a study of elite female water polo players by Varamenti et al. compared Specific Preparation (i.e., September), Competition phase (i.e., November and February) and Competition Phase after a taper period immediately prior to championships (i.e., April). and found the highest oxidative stress during the Competition phase [i.e., reduced TAC (November only) and increased TBARS], with the pre-championships taper resulting in recovered (i.e., same as Specific Preparation phase) anti-oxidative capacity (i.e., TAC), but continued elevation in pro-oxidant status (i.e., increased PC and TBARS). And though there was variation in sampling points and analysis methods, studies of professional soccer players all found elevations of oxidative stress during the Competition phase (i.e., in-season) relative to baseline (i.e., prior to training) or General Preparation phase (i.e., pre-season) values (Becatti et al., 2017; Le Moal et al., 2016; Ponce-Gonzalez et al., 2021; Silva et al., 2014). A potentially distinguishing factor is that for team sports the Pre-Compeition/Competition phase is extended (e.g., eight months long in the case of professional soccer) and the lines between Pre-Competition and Competition Phases are blurred because top level performance of the team is required during Pre-Competition (i.e., prior to playoffs/championships) in order to qualify for playoffs/championships. In the case of

individual sports such as running, much more focus can be placed on peaking performance for individual events [e.g., a single qualifying race (e.g., Olympic qualifiers) and a single championship race (e.g., Olympics)].

One universal constraint to previous research on seasonal alterations of redox balance is the limited number of sample points, as only two to five sample points were included throughout the competitive season for any given study, not allowing for more refined resolution in redox alterations. In addition, other than an evaluation of elite female waterpolo players (Varamenti et al., 2013), previous research on seasonal alterations of redox balance in elite athletes is exclusively on male athletes. Our is the first study to report alterations in redox balance across all 12 months of the competitive season for elite athletes of any discipline, including 177 sample points over nearly a five-year period for both male and female athletes. The significance of our findings is three-fold. First, this research supports the previous research that there are seasonal alterations in redox balance and that they are sport specific and likely tied to periods of intensified training (i.e., increases in relative training load). Second, these finding should both bring awareness to coaches and team managers to closely manage training during this early adaptation period anduse redox biomarkers during this period to objectively monitor individual responses to training. Finally, though female athletes in this sample had significantly higher pro-oxidant status (i.e., FORT), there was no difference in response to time-in-season by sex.

There are several limitations to this study. First, like most studies involving elite/professional athletes, this is a field study, with data collection yielding to training schedule to prioritize athlete performance. Where possible, athletes were tested in the AM in a fasted,

rested, and hydrated state, though due to training and racing schedules, this was not always the case. In addition, there was variability in the time interval between repeated samples from different athletes. Furthermore, though the timing of increased oxidative stress in our sample (i.e., General Preparation period), is typically a period where endurance track athletes experience high relative training load, specific training activities were not captured and therefore training load could not be directly assessed.

In conclusion, the main finding was that oxidative stress was elevated during pre-season training of elite endurance track athletes, particularly in December (i.e., General Preparatory training period), which was primarily driven by decreases in anti-oxidative capacity (i.e., FORD). Additionally, female elite endurance track athletes had significantly elevated oxidation levels (i.e., FORT) relative to male athletes, but there was no interaction effect of sex and time-in-season. These results,, in combination with recent work documenting the influence of oral contraceptive use on the redox balance of females athletes (Cauci et al., 2016; Quinn et al., 2021; Quinn et al., 2020), suggests that future work is needed to evaluate the impact of oral contraceptives on adaptive signaling. Notwithstanding, this work indicates that particular attention should be paid to redox balance early in the annual training cycle while athletes are re-adapting to higher training loads, even though the overall intensity of training is typically lower during this period. Additionally, target ranges should be individualized, accounting for sex and oral contraceptive use and dynamics of excursions outside of these ranges should be monitored so that training and nutrition can be actively managed to quickly restore redox balance.

Impact of altitude and on redox balance in elite endurance track athletes

Altitude training is a key component of elite endurance athlete training program because of the potential to increase oxygen carrying capacity but has also been shown to increase oxidative stress (Leon-Lopez et al., 2018; Pialoux, Mounier, Brown, et al., 2009) as well as rates of illness (Koivisto et al., 2019). However, PoC biomarkers of oxidative stress associated with altitude training of elite endurance track athletes has never been reported. The aim of this study was to determine if high altitude exposure affects PoC biomarkers of oxidative stress in elite endurance track athletes. The primary finding was that there was no significant effect of altitude on PoC biomarkers of oxidative stress over 26 days of training at altitude in this sample of elite endurance track athletes.

High altitude exposure and intensive exercise bouts have been shown to increase oxidative stress (Pena, El Alam, Siques, & Brito, 2022; Quindry et al., 2016), though study of the effect of altitude on redox biomarkers in elite athletes is limited. Redox balance is of particular interest during altitude training as pro-oxidant signaling drives both adaptation to exercise (Vargas-Mendoza et al., 2021) and erythropoietin (EPO) upregulation via hypoxia inducible factor (HIF-1α) subunit stabilization (Klumpen et al., 2017) and therefore optimal altitude stimulus may involve simultaneous increases in pro-oxidant and anti-oxidative status to drive the greatest adaptive response (i.e., increases in EPO and subsequently hemoglobin mass) (Leon-Lopez et al., 2018; Raberin et al., 2021). Work by Raberin et al. with elite swimmers not only reported increases in both pro-oxidant and anti-oxidative status during altitude training (Leon-Lopez et al., 2018), but also reported that the athletes with the most favorable redox balance were also the highest performing athletes in previous study (Rodriguez

et al., 2015). To complicate the matter, there is large individual variation in response to altitude with a long-standing discussion around what differentiates "responders" versus "non-responders" and whether or not this classification is a fixed trait (McLean, Buttifant, Gore, White, & Kemp, 2013; Nummela, Eronen, Koponen, Tikkanen, & Peltonen, 2021; Rusko et al., 2004). Specific mechanisms distinguishing altitude responders from non-responders is still largely unknown even though some individual differences have been identified to play a role, as both lowlanders (Sinha, Ray, Saha, Singh, & Tomar, 2009) and EIH athletes (i.e., athletes that experience exercise induced hypoxemia during sea level training) have shown greater oxidative stress response to altitude exposure (Raberin et al., 2021), with additional genetic (Sharma et al., 2021) and nutritional factors (Biuomy et al., 2020; Caris & Santos, 2019; Michalczyk, Czuba, Zydek, Zajac, & Langfort, 2016; Stellingwerff et al., 2019; Xie et al., 2020) likely playing a role. In the present study, there was no significant change in redox balance detected over 26 days of training at an altitude camp that consisted of eight (4 male and 4 female) elite endurance track athletes. However, there was some individual variation in response to altitude as three of the eight athletes surveilled did experience a single cogent elevation of oxidative stress balance (i.e., OSI) over the course of the altitude training camp, with the timing of this increase on three separate sample points (i.e., one each at day 12, 19 and 26) and therefore these alterations could not be materially differentiated from training related fluctuations. There was no hematology data such as EPO or hemoglobin mass reported therefore no assessment could be made to differentiate responders and non-responders in our sample. Individual alterations in FORT, FORD and OSI are shown in Figure 13, Figure 14 and Figure 15 in the Supplementary Data Section.

There were some notable limitations to this study. First, the sample size is small as only eight athletes attended the altitude training camp, which is an inherent limitation in studying homogeneous groups of elite athletes. Second there was no sampling of these athletes immediately before the altitude training camp for comparison, so a baseline value was calculated for each athlete using the individual mean from all sea level samples that had been collected. Nonetheless, there was a large number of samples from which to calculate these baseline values [i.e., an average of 38 sea-level samples per athlete (range: 6-83)], and in visual comparison of these calculated values to individual trends, the calculated values are believed to accurately represent baseline conditions. Third, the training of these athletes was being actively monitored and managed and therefore training loads may have been successfully adjusted to avoid a significant and/or continued disruption in redox balance. Fourth, though training may have been performed at higher altitudes, the base altitude for this training camp was approximately 2100 meters, which is in the lower end of the suggested training altitude range of 2000-2500 meters (Chapman et al., 2014), so a significant alteration in redox balance may have been detected if the altitude camp was performed at a higher base (i.e., living) altitude. And finally, there was no hematology data available to evaluate if there was an association between the three athletes that showed an elevation in oxidative stress balance and change in oxygen carrying capacity (e.g., increases in hemoglobin mass).

In conclusion, the main finding was that there was no significant effect of altitude on PoC biomarkers of oxidative stress over 26 days of training at high altitudes in this sample of elite endurance track athletes. Previous research on the effect of altitude training on elite athletes reported both an increase in pro-oxidant status (i.e., increased plasma nitrate levels and LPO) and an increase in anti-oxidative capacity (i.e., GSSG/GSH ratio), leading to

concluding that the overall effect on oxidative stress balance was unclear (Leon-Lopez et al., 2018). Further research on the effect of altitude exposure on elite athletes is warranted, preferably utilizing PoC biomarkers along with previously efficacious biomarkers (i.e., plasma nitrates, LPO and GSSH/GSH) in the same sample of athletes to investigate biomarker specific alterations in pro-oxidant status, anti-oxidative capacity and overall oxidative stress balance. In addition, a concurrent assessment of changes in EPO and hemoglobin mass along with oxidative balance would provide better clarity on the association between alterations in redox balance and the intended physiological response to altitude exposure.

Impact of COVID-19 mRNA vaccination on redox balance and inflammation

Both mRNA COVID-19 vaccination (Ntouros et al., 2022) and SARS-CoV2 infection (Schmitt, Labdouni, Soulimani, Delamare, & Bouayed, 2022; Wieczfinska, Kleniewska, & Pawliczak, 2022) and have been associated with increased levels of oxidative stress, as well as the oxidative stress related conditions such as myocarditis and pericarditis (Dursun, Saricam, Sariyildiz, Iscanli, & Cantekin, 2022), however neither the effect of mRNA vaccination on athletes, nor PoC biomarkers of oxidative stress have been reported. The aim of this study was to determine if there is a change in PoC biomarkers of oxidative stress after mRNA COVID-19 vaccination of elite endurance track athletes. The primary finding was that COVID-19 mRNA vaccination [SPIKEVAX™ (mRNA-1273), Moderna Co., Cambridge, MA, USA] transiently increased oxidative stress (i.e., OSI) six days after the first vaccine dose, with a reduction in anti-oxidative capacity (i.e., FORD) that persisted throughout the inter-dose period. The secondary finding was that a stark but transient rise in a marker of inflammation

(i.e., hsCRP) was detected in the first 6 days after the first COVID-19 mRNA vaccine dose in some by not all athletes.

This is the first study to report oxidative stress after mRNA vaccination in athletes, however these results showing a transient increase in oxidative stress correspond to available data in non-athlete samples. Ntouros et al. investigated blood plasma biomarkers of oxidative stress in non-immunocompromised older individuals (80-96 years old) and healthy hospital personnel (27-44 years old) after mRNA vaccination (Comirnaty® (BNT162B2), Pfizer/BioNTech, New York, NY, USA) and found a reduction in anti-oxidative capacity (i.e., reduced GSH/GSSG ratio) 24 hours after the first dose, with levels returning to pre-vaccine baseline levels at the next two sample points (i.e., 14 days post both first and second vaccine doses) (Ntouros et al., 2022; Ntouros et al., 2021). In addition, these authors reported increased pro-oxidant status (i.e., DNA damage accumulation) in both non-immunocompromised older individuals (80-96 years old) and healthy hospital personnel (27-44 years old) with the same time-course profile (i.e., increased at 24 hours after the first mRNA vaccine dose, with levels returning to pre-vaccine baseline levels at 14 days post both first dose and second vaccine doses) (Ntouros et al., 2022). Furthermore, the same group of healthy hospital personnel were also monitored after conventional viral-vector influenza vaccine (VaxigripTetra™, inactivated Influenza vaccine, Sanofi Pasteur, Paris France) and a similar reduction in anti-oxidative capacity (i.e., GSH/GSSG) was observed, providing evidence that the alteration in redox balance is likely due to the intended immunological response (Henrik et al., 2022) and not specific to mRNA vaccination (Ntouros et al., 2021). Conversely, oxidative stress outside the blood compartment was evaluated after mRNA vaccination by Olana et al. (i.e., in seminal plasma) and no elevation of oxidative stress [i.e., colorimetric determination of reactive oxygen

metabolites (d-ROM test)] was detected (Olana et al., 2022) despite the negative effect of SARS-CoV-2 infection on gametogenesis and risk of male infertility (Omolaoye, Jalaleddine, Cardona Maya, & du Plessis, 2022). Due to the recent approval [i.e., FDA emergency use approval for the first mRNA vaccine (BNT162B2, and later named Comirnaty®] was on December 11th, 2020), there is a paucity of data on alterations in redox balance following mRNA vaccination, yet COVID-19 infection has been intensely studied and oxidative stress is widely believed to play a prominent role in pathogenesis (Martinez Mesa et al., 2021) and severity (Cakirca, Damar Cakirca, Ustunel, Torun, & Koyuncu, 2021; Pincemail et al., 2021). And though there is still no clear etiology of the disease (Yang, Ma, Tjong, Stern, & Chiu, 2021), further evidence of an oxidative aspect is that anti-oxidative treatments such as melatonin, glutathione and the glutathione precursor N-acetylcysteine have shown promise in reducing disease severity (Cazzola, Rogliani, Salvi, Ora, & Matera, 2021; De Flora, Balansky, & La Maestra, 2020; Farnoosh et al., 2022; Zhou, Yang, Huang, & Chen, 2020).

Elevated C-Reactive Protein (CRP) levels have also been associated with mRNA vaccination induced clinical conditions of myocarditis (Goyal et al., 2022) and myopericarditis (Dursun et al., 2022), as well as COVID-19 infection severity (El-Hefnawy et al., 2022; Keshani et al., 2022; Pincemail et al., 2021; Saha, Thakuria, & Swami, 2022), however alterations in CRP after mRNA vaccination in non-clinical cases has not been reported. Among some, but not all athletes, one day after the administration of the first mRNA vaccine dose there was an elevation in hsCRP, which quickly resolved. A retrospective study by Ostrowski et al. monitored CRP levels before and after SARS-CoV-2 vaccination (both mRNA and conventional viral-vector) and did not find any change in CRP levels, however their first post vaccination sampling point was not until eleven days after the 1st vaccination dose (Ostrowski

et al., 2021). Although the previous infection history of our athletes is unknown, the differential CRP response among athletes could have been due to prior COVID-19 infection, as both CRP and IgG antibody kinetics are increased with prior antigen exposure (Gianfagna et al., 2022). The fact that CRP quickly resolved post mRNA vaccination in the present study is in stark contrast to COVID-19 infection, where reports of persistent elevation of CRP and other markers of inflammation in studies evaluating up to 300 days post infection (Gianfagna et al., 2022). The pathophysiology of continued inflammatory marker elevation post COVID-19 infection in some patients is not resolved, however recent data suggests that elevation of SARS-CoV-2 specific T cells may play a role (Littlefield et al., 2022).

The primary practical significance of our results is that athletes should likely reduce training load in the week following the first mRNA vaccine dose to account for increased oxidative stress and mobilization of the immune response (Bergamaschi et al., 2021). This time frame also corresponds to the acute symptomology in elite athletes reported by Hull et al., as more than 80% of the athletes in their sample of 127 athletes (70 male and 57 female, 27.5±4.9 years old) experienced side effects after the first Comirnaty® vaccine dose, with all symptoms (other than residual fatigue) resolving within the first 7 days (fatigue resolved in all athletes by day 9) (Hull, Wootten, & Ranson, 2022). Historically elite athletes are hesitant in performing any activity that may affect training load, including conventional viral-vector vaccination (F & J, 2021), however the protective effect of SARS-CoV-2 vaccination far outweighs acute training load consideration as a study of COVID-19 infection in elite athletes reported that 25% of athletes studied had not fully returned to training a full month after the onset of infection (Hull, Wootten, Moghal, et al., 2022). As far as training considerations before vaccination, Stenger et al. reported that there was no difference in immune response or side

effects of athletes who received the influenza vaccine (Influsplit™ Tetra, GlaxoSmithKline Gmbh & Co, Munich, Germany) two hours after training versus 24-26 hours after training (Stenger et al., 2020), indicating a reduction of training prior to mRNA vaccination is likely unnecessary. An additional consideration for athletes may be the use of nutritional countermeasures; since the primary change in oxidative stress was due to a reduction in anti-oxidative capacity (i.e., FORD), a nutritional intervention may be particularly beneficial before and/or during this period. Nutritional interventions have not been studied in conjunction with mRNA vaccination, though there is evidence that anti-oxidative whole-food nutrition and supplementation could decelerate the aggressive and lethal development of COVID-19 (Chavarria et al., 2021; Iddir et al., 2020; Notz et al., 2021; Trujillo-Mayol et al., 2021). Nutritional interventions with athletes to minimize training induced alterations in redox balance have shown effectiveness, though the effect on performance has been mixed (de Oliveira, Rosa, Simoes-Ambrosio, Jordao, & Deminice, 2019; Koivisto et al., 2019; Pastor & Tur, 2019) as superabundance of nutritional antioxidants has been reported to blunt the adaptive process (Mason et al., 2020). There is an exhaustive body of antioxidant research, which is beyond the scope of this discussion, but the current consensus seems to be that whole-food ingestion of antioxidants is beneficial and will not exceed recommended levels from a micronutrient standpoint (Koivisto et al., 2018), albeit there may be upper limits of beneficial macronutrient intake as there are typically optimal ranges associated with individual sport disciplines (Malsagova et al., 2021).

In spite of the fact that our results only showed significant alteration in inflammation and redox balance after the first mRNA vaccination dose, a brief reduction in training load after the second vaccine dose may also be warranted as mRNA vaccination has been shown to

temporarily elevate a number of pro-inflammatory cytokines and chemokines, primarily after the second dose, particularly in individuals without prior COVID-19 infection (Gianfagna et al., 2022). A study by Bergamaschi et al. specifically showed greater elevations in inflammatory cytokines IFN-γ and IL-6 of and chemokine IP-10/CXCL10 the day after the second BNT162b2 vaccine dose, relative to the first vaccine dose (Bergamaschi et al., 2021). This acute inflammatory response is likely beneficial in terms of immune response as greater elevation of inflammatory markers was also associated with higher resulting IgG titers (Bergamaschi et al., 2021; Gianfagna et al., 2022), however it is also linked to vaccination induced myocarditis and pericarditis (Dursun et al., 2022), as well as persistent elevation of inflammatory markers associated with the pathogenesis of a multitude of diseases (Antos & Savant, 2022; Karimabad et al., 2021; Khanijou, Zafari, Coughlan, MacIsaac, & Ekinci, 2022; Liberale et al., 2022; Liou, Yoon, Maher, & Chwalisz, 2022; Petreski, Piko, Ekart, Hojs, & Bevc, 2021). There is no published data on redox or CRP alterations after a booster dose (i.e., any dose beyond the completion of the primary vaccine series) of mRNA vaccine, but studies in other immunological response suggest that the response will be similar to the second vaccine dose due to the priming effect of prior exposure to the spike protein antigen (Sablerolles et al., 2022; Tamburro et al., 2022). Together these data suggest that it would be prudent for athletes to reduce training load post second vaccination dose for a minimum of 48 hours to account for the semi-acute physiological stress associated with the intended immune response.

There are several limitations to the present study. First, and most notably timing of vaccine availability happened to coincide with an altitude training camp, and these athletes were vaccinated at altitude, therefore an additional effect of altitude cannot be ruled out. However, as documented in the previous section, *Impact of altitude and on redox balance in*

elite endurance track athletes, the result of our data and analysis was that altitude exposure did not alter redox balance in an overlapping group of elite endurance track athletes. The results from this study are in line with previous vaccination literature, yet because the additive effect of altitude exposure during mRNA vaccination cannot be accounted for and therefore further study is warranted to confirm these results. Second, the sample size is small as only nine athletes were available for study. This in an inherent limitation in studying elite athletes. Previous work indicates that vaccination induced antibody titers of elite athletes is not different than the general population (Gartner & Meyer, 2014) and therefore future study of the immunological response to mRNA vaccination involving a broader, more accessible demographic would be of value. Third, there was variation in the timing of sample collection relative to vaccination between athletes necessitating the use of mean values for comparison, which may have impaired the precision in the timing of the alterations in redox balance and hsCRP found. However, because these changes were shown to be within the first week after vaccination, it would be beneficial for future study to intensify sampling throughout that period to provide further insight into the dynamics of the physiological response.

In conclusion, the main finding of the present study was that PoC markers of oxidative stress were significantly elevated in the first six days after the first Comirnaty® mRNA vaccination dose, but quickly resolved. The secondary finding was that the inflammatory marker of hsCRP was significantly elevated the day after the first vaccine dose, with notable individual variation - some athletes showing a marked increase (i.e., greater than three-fold), whereas other athletes were unaffected. In combination with previous work, the practical takeaway from this study is that athletes and coaches should be mindful of training load in the 2-7 days post each mRNA vaccine dose to account for increased physiological stress from the

indented immunological response, along with consideration of anti-oxidative nutrition throughout the inter-dose period.

Summary

The studies detailed in this dissertation were performed to address three specific aims and one additional exploratory aim. The aims of these studies were:

1. Compare PoC biomarkers of oxidative stress in elite endurance track athletes with published data for disease states and sedentary controls.
2. Investigate alterations in the redox balance of elite endurance track athletes over the course of the competitive year using PoC biomarkers of oxidative stress.
3. Determine if high altitude exposure effects PoC biomarkers of oxidative stress in elite endurance track athletes.
4. Determine if there is a change in PoC biomarkers of oxidative stress after mRNA COVID-19 vaccination of elite endurance track athletes.

The primary findings of these studies were:

1. The primary finding was that mean oxidative stress levels in elite endurance track athletes are lower than reported disease states but not significantly different than healthy controls or other characterized athlete populations (except for FORT values of Elite UK Endurance Athlete, which were significantly higher). Additionally, it was found that although levels were not sustained, elite endurance athletes did experience transient elevations of oxidative stress into ranges associated with disease states, particularly in terms of reduction of anti-oxidative capacity (i.e., FORD).

2. The primary finding was that oxidative stress balance (i.e., OSI) was elevated during pre-season training of elite endurance track athletes, particularly in December relative to every other month of the year except February, March, and April. This increase in oxidative stress was chiefly driven by decreases in anti-oxidative capacity (i.e., FORD). Additionally, it was found that pro-oxidant status (i.e., FORT) was elevated in females relative to males throughout the competitive year, with no differential effect of time-in-season between sexes.
3. The primary finding was that there was no significant effect of altitude on PoC biomarkers of oxidative stress in this sample of elite endurance track athletes.
4. The primary finding was that COVID-19 mRNA vaccination [SPIKEVAX™ (mRNA-1273), Moderna Co., Cambridge, MA, USA] transiently increased oxidative stress balance (i.e., OSI), chiefly due to a decrease in anti-oxidant defense (i.e., FORD) in the first 6 days after the initial vaccine dose in elite endurance track athletes. Additionally, it was found that COVID-19 mRNA vaccination briefly increased inflammation levels (i.e., hsCRP) the day after the initial vaccine dose in some by not all elite endurance track athletes.

In conclusion, the overall results of this dissertation demonstrate that PoC redox biomarkers are sensitive to the semi-acute stressors of training phase and vaccination and provide a valuable objective means to monitor the physiological condition and resilience of athletes, especially for high level programs where professional longevity depends on continued health, minute fluxuations in athlete performance are meaningful, and sport scientists are available to interpret the data from this pioneering bio-analytical method.

Chapter 5

Supplementary Data

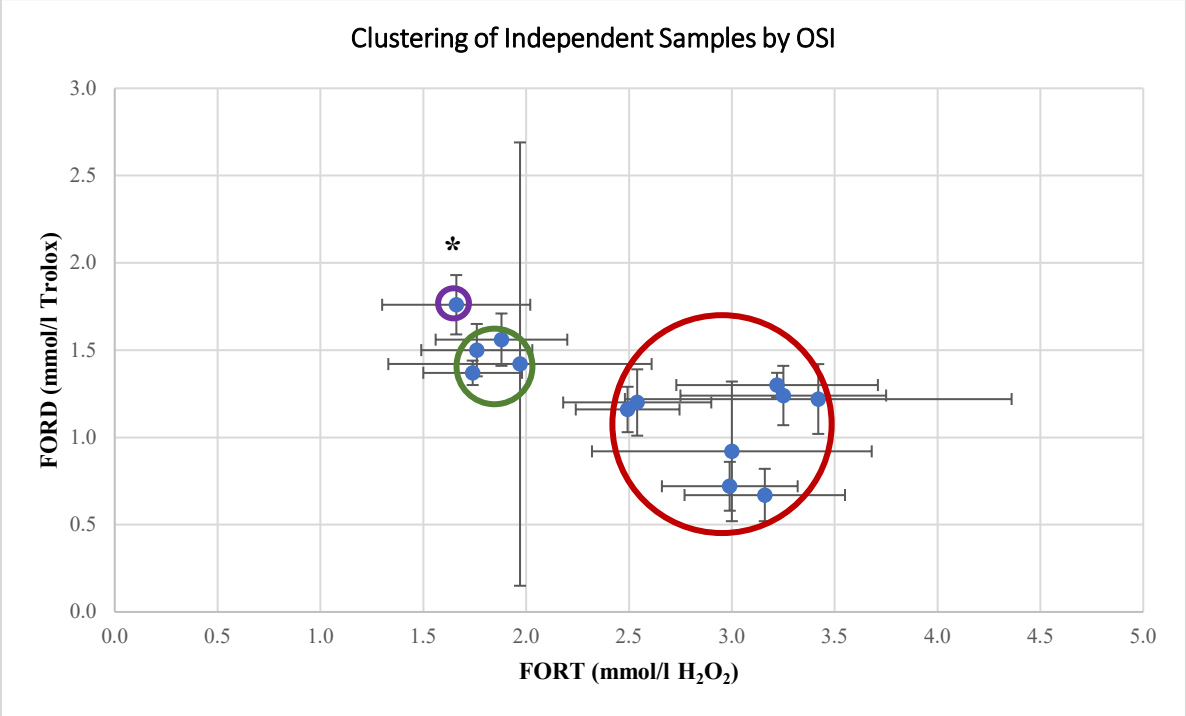

Figure 11. Two-dimensional clustering of redox values by oxidative stress index (OSI = FORT/FORD) across sample groups. Data are mean ±SD. Samples in the red and purple circles are significantly different than samples in the green circle. Green circle indicates not significantly different from each other but are significantly different from the cluster in the red circle, $p<0.05$. * Indicates FORD only.

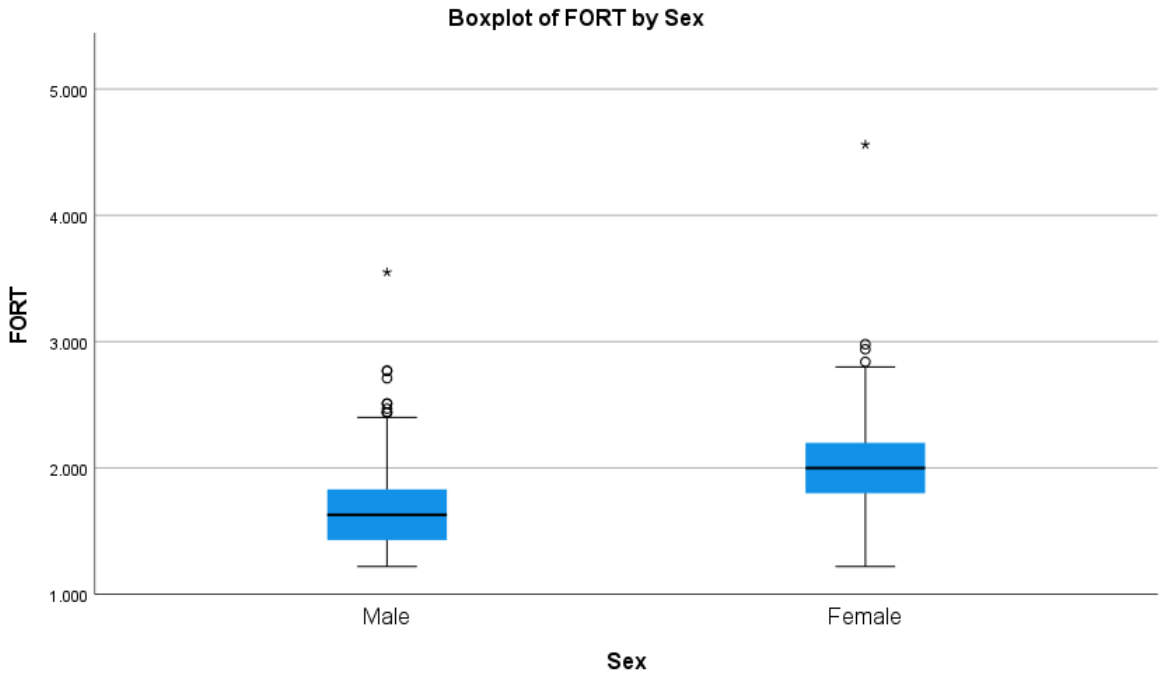

Figure 12. Boxplot of FORT values by sex. Circles indicate outlier datapoints and *indicates extreme outlier datapoints. There was a significant effect of sex, p=0.002.

Table 7: Redox alterations during altitude training camp. Altitude sample points are number of days at altitude. Sea level data are mean values for each athlete and all other data are individual sample data.

Athlete	M/F	Test	Sea Level*	Altitude +5	Altitude +12	Altitude +19	Altitude +26
		FORT	1.93	1.22	2.11	2.14	2.11
1	**Male**	**FORD**	1.73	1.67	1.62	1.63	1.57
		OSI	1.12	0.73	1.30	1.31	1.34
		FORT	1.63	1.57	1.57	1.30	1.35
2	**Male**	**FORD**	1.54	1.75	1.76	1.71	1.29
		OSI	1.06	0.90	0.89	0.76	1.05
		FORT	1.65	1.94	1.54	1.60	1.63
3	**Male**	**FORD**	1.39	1.17	1.38	1.48	1.32
		OSI	1.19	1.66	1.12	1.08	1.23
		FORT	2.09	1.66	1.60	1.70	1.40
4	**Female**	**FORD**	1.61	1.76	1.51	2.56	1.14
		OSI	1.30	0.94	1.06	0.66	1.23
		FORT	1.94	N/Av	1.63	1.70	1.90
5	**Male**	**FORD**	1.40	N/Av	1.40	1.39	1.41
		OSI	1.39	N/Av	1.16	1.22	1.35
		FORT	2.16	2.27	2.00	2.20	2.14
6	**Female**	**FORD**	1.42	1.72	1.62	1.17	0.73
		OSI	1.52	1.32	1.23	1.88	2.93
		FORT	1.90	1.83	4.56	2.07	1.94
7	**Female**	**FORD**	1.47	1.97	1.66	1.32	N/Av
		OSI	1.29	0.93	2.75	1.57	N/Av
		FORT	1.46	1.76	1.57	2.04	1.40
8	**Male**	**FORD**	1.25	1.52	1.77	0.82	1.38
		OSI	1.17	1.16	0.89	2.49	1.01

Table 8: Redox and inflammation (hsCRP) alterations throughout vaccination period. Data are individual sample points. ID indicates assigned athlete ID number and M/F indicates sex.

			Timepoint (Day)													
ID	**M/F**	**Test**	**D1 -63±3**	**D1 -56±3**	**D1 -49±3**	**D1 -43±4**	**D1 -36±4**	**D1 -25±8**	**D1 -6±3**	**D1 +1±0**	**D1 +6±1**	**D1 +12±1**	**D1 +19±1**	**D1 +26±1**	**D2 +6±2**	**D2 +19±6**
3	**M**	**FORT**	1.80	1.60	1.72	1.57	1.40	1.54	1.39	N/Av	1.90	1.54	1.22	1.23	1.22	1.57
		FORD	1.66	1.13	0.79	1.62	1.36	1.33	1.30	N/Av	1.06	0.77	0.72	0.75	0.74	1.08
		OSI	1.08	1.42	2.18	0.97	1.03	1.16	1.07	N/Av	1.79	2.00	1.69	1.64	1.65	1.45
		hsCRP	0.84	1.50	1.00	1.18	0.87	1.04	1.20	N/Av	4.44	1.12	0.96	N/Av	2.25	0.96
5	**M**	**FORT**	N/Av	N/Av	N/Av	N/Av	N/Av	N/Av	1.92	1.80	2.71	1.87	2.47	2.77	2.04	1.83
		FORD	N/Av	N/Av	N/Av	N/Av	N/Av	N/Av	1.38	0.88	1.03	1.14	0.83	0.99	1.28	1.23
		OSI	N/Av	N/Av	N/Av	N/Av	N/Av	N/Av	1.45	2.05	2.63	1.64	2.98	2.80	1.59	1.49
		hsCRP	N/Av	N/Av	N/Av	N/Av	N/Av	N/Av	0.87	5.12	5.25	N/Av	N/Av	4.19	0.81	0.84
6	**F**	**FORT**	1.60	1.67	2.14	2.00	2.20	2.23	2.17	N/Av	2.23	2.20	1.80	1.90	2.47	2.23
		FORD	1.68	1.65	1.13	1.66	1.42	1.75	1.07	N/Av	1.03	1.20	1.04	0.83	1.07	1.81
		OSI	0.95	1.01	1.89	1.21	1.55	1.27	2.03	N/Av	2.17	1.83	1.73	2.29	2.31	1.23
		hsCRP	0.86	0.80	0.86	0.80	0.83	0.77	0.80	N/Av	0.92	0.84	N/Av	N/Av	1.46	0.83
7	**F**	**FORT**	N/Av	N/Av	2.34	2.00	2.17	2.00	2.04	2.07	2.14	1.87	1.45	1.22	1.90	2.23
		FORD	N/Av	N/Av	1.87	0.92	1.87	1.31	1.85	0.76	0.78	1.24	0.58	1.07	1.36	1.32
		OSI	N/Av	N/Av	1.25	2.17	1.16	1.53	1.10	2.72	2.74	1.51	2.50	1.14	1.40	1.69
		hsCRP	N/Av	N/Av	0.84	0.94	0.86	0.81	0.79	N/Av	5.88	1.20	N/Av	0.94	0.92	1.07
9	**F**	**FORT**	2.04	2.34	2.17	2.47	2.37	N/Av	N/Av	1.54	2.14	1.22	1.40	1.60	1.83	1.94
		FORD	1.70	1.21	1.32	1.49	1.41	N/Av	N/Av	1.30	1.28	0.51	0.95	0.94	1.36	1.25
		OSI	1.20	1.93	1.64	1.66	1.68	N/Av	N/Av	1.18	1.67	2.39	1.47	1.70	1.35	1.55
		hsCRP	0.77	0.92	10.30	0.84	0.94	N/Av	N/Av	5.09	0.87	N/Av	N/Av	0.89	0.83	0.94
10	**M**	**FORT**	1.73	1.47	1.52	1.70	1.22	1.63	1.80	1.30	1.47	1.63	1.36	N/Av	1.66	1.66
		FORD	1.71	0.96	1.13	1.79	1.85	1.20	1.90	0.97	1.10	1.08	1.54	N/Av	0.99	1.28
		OSI	1.01	1.53	1.35	0.95	0.66	1.36	0.95	1.34	1.34	1.51	0.88	N/Av	1.68	1.30
		hsCRP	9.16	2.70	N/Av	4.64	2.60	4.23	4.03	13.75	10.22	N/Av	N/Av	N/Av	15.12	2.58
11	**M**	**FORT**	1.50	1.76	1.66	N/Av	1.83	1.54	1.80	N/Av	1.63	1.22	1.47	2.44	1.66	1.70
		FORD	1.92	1.86	1.47	1.73	0.84	1.10	0.98	N/Av	0.77	1.33	1.03	0.93	1.50	1.58
		OSI	0.78	0.95	1.13	N/Av	2.18	1.40	1.84	N/Av	2.12	0.92	1.43	2.62	1.11	1.08
		hsCRP	0.81	0.87	0.86	0.91	0.83	0.94	5.00	0.96	N/Av	N/Av	N/Av	1.96	0.91	0.91
12	**M**	**FORT**	1.36	1.23	1.22	1.50	1.36	1.23	1.22	1.22	1.54	1.57	1.33	N/Av	N/Av	1.23
		FORD	1.47	1.77	1.79	1.73	1.81	1.74	1.51	0.98	1.55	1.35	1.55	N/Av	1.02	1.20
		OSI	0.93	0.70	0.68	0.87	0.75	0.71	0.81	1.25	0.99	1.16	0.86	N/Av	N/Av	1.03
		hsCRP	0.94	0.91	0.80	0.92	0.84	0.91	0.92	1.02	0.98	N/Av	N/Av	N/Av	1.31	0.92
13	**M**	**FORT**	2.04	1.54	2.11	N/Av	1.54	1.63	1.30	1.63	1.76	1.23	1.26	N/Av	2.11	1.73
		FORD	1.69	1.84	1.33	N/Av	1.53	1.41	0.95	1.43	0.83	1.19	1.18	N/Av	1.18	1.55
		OSI	1.21	0.84	1.59	1.01	1.16	1.37	N/Av	1.14	2.12	1.03	1.07	N/Av	1.79	1.12
		hsCRP	0.91	0.84	0.87	N/Av	1.00	0.87	0.96	0.98	1.02	0.96	N/Av	N/Av	2.21	1.04

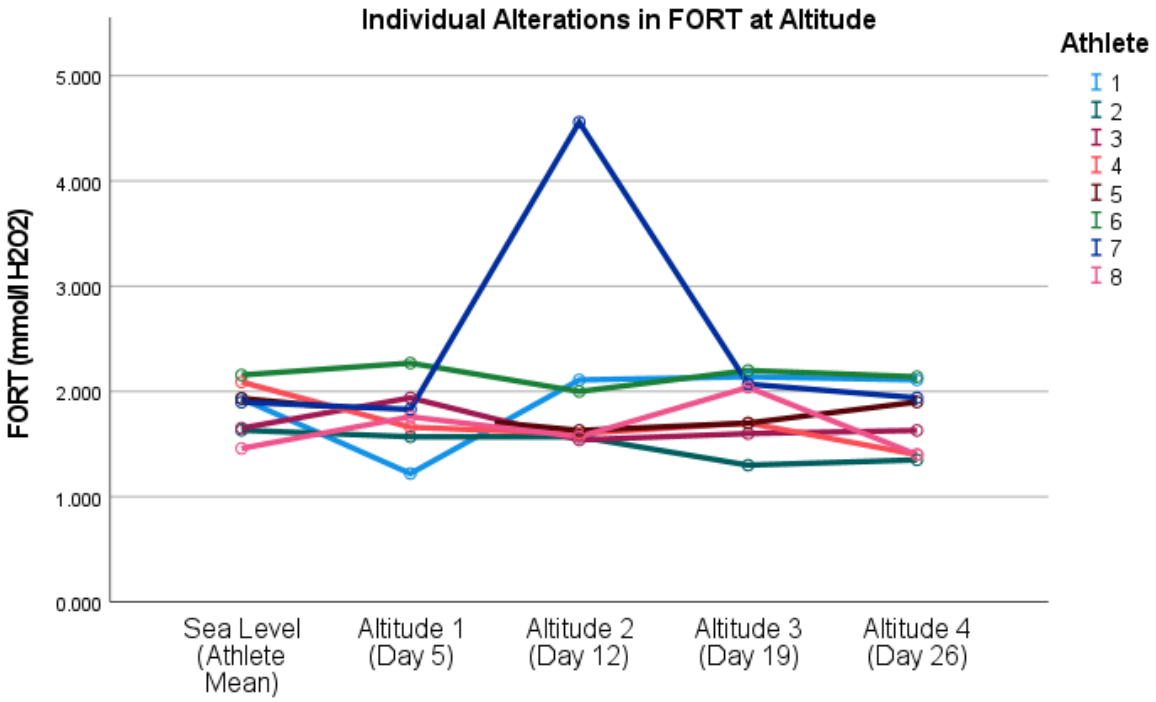

Figure 13. Individual alterations in FORT over duration of altitude training camp.

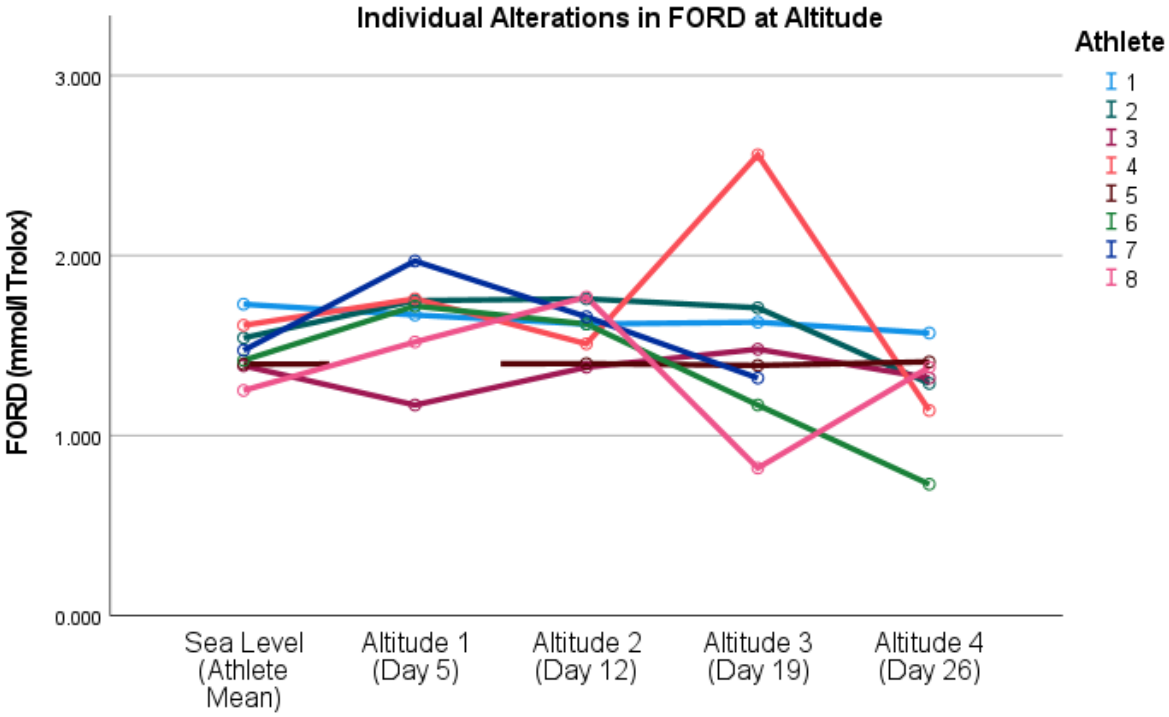

Figure 14. Individual alterations in FORD over duration of altitude training camp.

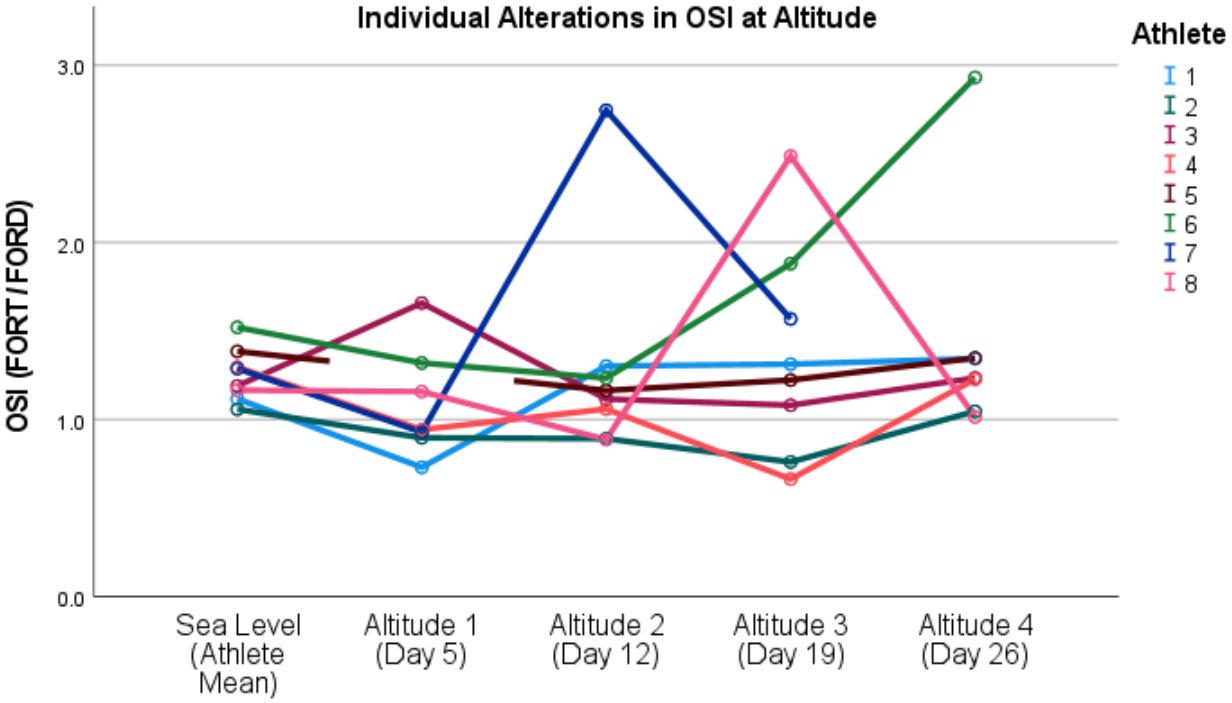

Figure 15. Individual alterations in OSI over duration of altitude training camp. *Sea level values are individual athlete mean values.

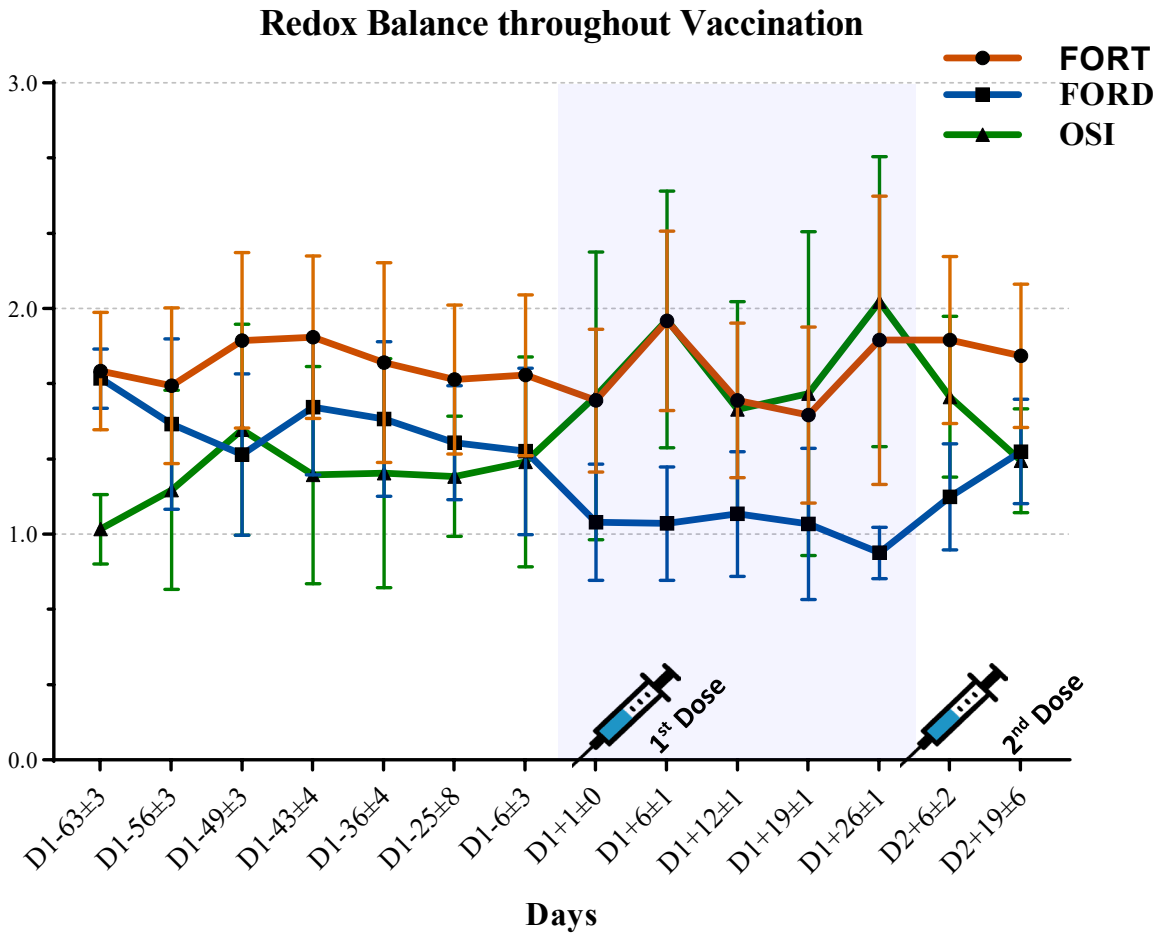

Figure 16. Mean alterations in redox balance throughout vaccination period. No significant alterations in FORT values post vaccine. Significant alterations in FORD and OSI with vaccination plus altitude are shown in Figures 11 and 12, respectively.

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