Title: Increased Oxidative Stress in Injured and Ill Elite International Olympic Rowers

Submission type: Original investigation.

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Keywords: biomarkers, athlete, monitoring, endurance

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Abstract word count: 250

Text only word count: 3547

24 References

2 Figures

2 Tables

Abstract (250)

Identifying strategies that reduce the risk of illness and injury is an objective of sports science and medicine teams. No studies have examined the relationship between oxidative stress (OS) and illness or injury in international athletes undergoing periods of intensified training and competition. **Purpose:** We aimed to identify relationships between illness, injury and OS. **Methods:** A longitudinal, observational study of elite male rowers (n=10) was conducted over 18-weeks leading into World Championships. Following a recovery day and a 12-hour fast, hydroperoxides (FORT) and total anti-oxidant capacity (FORD) were measured in venous blood, with the ratio calculated as the oxidative stress index (OSI). At all study time points, athletes were independently dichotomized as ill or not ill, injured or not injured. OS data were compared between groups using independent t-tests. A Cox proportional hazard model was used to assess the association of OS with injury and illness while adjusting for age and body mass index. **Results:** FORD was lower ($p<0.02$) and OSI was higher ($p<0.001$) with illness than without illness. FORT and OSI were higher with injury than without injury ($p<0.001$). FORD exerts a protective effect on illness with 0.5 $mmol \cdot L^{-1}$ increase related to a 30.6% illness risk reduction ($p=0.014$), and OSI exerts a harmful effect on illness risk with a 0.5 unit increase in OSI related to an 11.3% increased risk ($p=0.036$). **Conclusion:** OS is increased in injured and ill athletes. Monitoring OS may be advantageous in assessing recovery from, and in reducing injury and illness risk given the association.

Introduction

Loss of training time due to illness and injury, is a major determinant of performance goal success or failure for elite international athletes competing in individual Olympic sports[1]. Exceedingly high training loads and/or spikes in training load are a recognised risk factor for injury and illness, and fatigue[2]. Furthermore, illness and accumulating fatigue may result in the development of chronic underperformance[3]; all of which can derail the athlete's season and may finish careers. Finding ways to reduce the risk of injury and illness is therefore a primary objective of the high-performance support team.

Oxidative stress (OS), historically and simply defined as a disturbance in the pro- to anti-oxidant balance in favour of the former[4], is evident in athletes diagnosed with overtraining syndrome (OTS[5,6]). Indeed, increases in biomarkers of OS correlate strongly with increases in training volume [7,8]. Furthermore, elite athletes designated as "impaired performers" had a twofold greater total peroxide load (reported in H_2O_2 equivalents) and were more prone to infections [9]. Such findings suggest that the longitudinal monitoring of OS biomarkers in the elite endurance athlete may provide an indicator of excessive training load, increased risk of illness and injury, and therefore allow for improved optimisation of workload [10].

International rowers are exposed to high volume training and are at a higher risk of injury than many non-contact sports, and some contact sports [11]. However, due to the non-contact nature of rowing, competition periods may pose less risk for injury; rowing being one of the sports with the lowest incidence of injury at the Rio Olympic Games [12]. In contrast, competition periods pose a greater risk of illness in elite athletes [13,14]. To the authors knowledge no studies have investigated the relationship between OS biomarkers and injury in athletes, and only one study has investigated the relationship with illness [9].

We have previously shown the clinical point-of-care OS test, known as free oxygen radicals test (FORT; a measure of hydroperoxides) and free oxygen radicals defence (FORD; a measure of plasma antioxidant capacity), to be repeatable [15], clinically useful in elite sport [6,16], and possess validity in terms of capturing acute changes in OS in elite endurance athletes [17]. In this observational study we analysed longitudinal data from elite international Olympic rowers. Rowing is a sport in which some of the largest physiologically relevant changes in OS have been reported [18]; reviewed in Lewis et al. [10], and we have previously shown evidence of substantial OS in an elite international rower diagnosed with unexplained under performance syndrome/overtraining syndrome using the FORT and FORD [6].

The aim of the present study was to identify the relationship between changes in OS biomarkers and injury and illness incidence during a competitive phase of the season in a prospective, longitudinal study of elite international rowers.

Methods

Subjects

Eight openweight (Age 26.9 ± 2.2 years, height 192.3 ± 3.1 cm, weight 93.1 ± 4.9 kg) and two lightweight (Age 28.6 ± 0.2 years, height 186.0 ± 1.4 cm, weight 73.3 ± 0.4 kg) male rowers (including World and Olympic medalists) were recruited to participate and provided written informed consent. Athletes were free living and attending a national training centre, were not taking any medications and were subject to United Kingdom Anti-doping controls and testing procedures. Data were collected weekly at the same time of day and included the following: oxidative stress via the point of care blood test, prescribed training volume and intensity and subjective assessments of wellness and sleep quality. All procedures were approved by the Internal Review Board of the English Institute of Sport. All athletes were tested in the competition phase of the annual cycle up until the World championships, including the World Cup series and two altitude training camps ranging from 2,000-2,300m. Athletes were classified as injured and ill by the medical staff, and for reasons of confidentiality, the specific details of the injuries and illnesses are not disclosed.

Design

This was a longitudinal observational study to establish the relationship between biomarkers of oxidative stress, injury and illness. Over the course of 122 days, 15 repeated measurements of oxidative stress were taken from these rowers from venous blood samples, plasma hydroperoxides and plasma antioxidant defence, along with the ratio of the two, the oxidative stress index (OSI). Testing was carried out between 6 a.m. and 9 a.m. in a fasted, hydrated, and rested state. In the 24 hours prior to testing, training was kept to either an aerobic training day of low to moderate intensity or a rest day. We present simple summaries of all biomarkers across illness and injured status, not adjusted for repeated measures. Each illness and injury was diagnosed and documented by the sports medicine team in the environment on the day of testing. In total there were eight episodes of illness in six of the rowers across the study period, of which three required antibiotics for treatment.

Methodology

Blood sampling for oxidative stress tests

Venous blood samples were taken from an antecubital vein using a 5 ml lithium heparin vacutainer tube (BD system; New Jersey, USA), with 50 μL and 20μL of blood transferred into heparinized capillary tubes for the analysis of FORD and FORT respectively, in line with the manufacturer's instructions (Callegari SpA, Catellani Group, Parma, Italy). Intra- and inter assay coefficients of variation for FORT and FORD were < 5% and 7%, respectively.

FORT assay

We have described the details of the assay previously [15]. Briefly, reactive oxygen species (ROS) activity was determined downstream through the measurement of hydroperoxides via the FORT test. FORT is a colourimetric assay based on the capacity of transition metal ions (Fe^{3+}

/Fe^{2+}) to catalyze the breakdown of hydroperoxides (R-OOH) into derivative radicals [alkoxyl (R-O•) and peroxyl radicals (R-OO•)] within the biological sample. The application of an acidic buffer to the 20μL blood sample, releases the transition metals from associated proteins, which react with the hydroperoxides present in the sample, producing the alkoxyl and peroxyl radicals. The derivative radicals are trapped through the addition of a buffered chromogen (reagent; an amine derivative, $CrNH_2$) and develop into a radical cation in a linear based reaction at a controlled temperature of 37°C, photometrically detectable at 505nm.

The intensity of the sample colour correlates with the quantity of radical compounds and therefore the concentration of hydroperoxides in the biological sample, according to Lambert-Beer's law. The results are expressed as equivalent concentrations of H_2O_2 mmol·L^{-1}.

FORD assay

The FORD test (Callegari, Catellani, Italy) determines the presence of plasma antioxidants via a colourimetric assay based on the capacity of the sample to reduce a preformed radical cation. In the presence of an acidic buffer and a suitable oxidant ($FeCl_3$), the chromogen that contains 4-amino-N,N-diethylaniline sulfate forms a stable and coloured radical cation, photometrically detectable at 505 nm. The antioxidant compounds present in the plasma sample reduce the radical cation of the chromogen, quenching the colour, and causing a discolouration of the sample, proportional to the concentration of antioxidants present. The absorbance values generated are compared to standard curves derived from Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a derivative of vitamin E with enhanced water solubility. FORD values are reported as Trolox equivalents, mmol·L^{-1}, linearity ranged from 0.25 to 3.0 mmol·L^{-1} Trolox.

Screening blood tests

Additional blood draws via the antecubital vein were conducted on two occasions, with 3 x 5 ml venous blood samples collected in a serum separator (SST) and 1 x 5 ml blood sample via an EDTA (ethylenediamine tetraacetic acid) vacutainer tube (BD system; New Jersey, USA). Full blood counts, iron status (Ferritin, Iron, Transferrin saturation), thyroid function (Thyroid Stimulating Hormone), nutritional status (vitamin C, vitamin D, alpha-tocopherol, and the carotenoids; lutein, alpha-carotene and beta-carotene), muscle damage and inflammation (Creatine Kinase, C-Reactive Protein), were measured at the beginning of the study period and again prior to the final training prior to the world championships.

We have previously described the methodology and assay performance of the aforementioned venous and capillary biomarkers [15].

Training Assessment

Training was prescribed by coaching staff in accordance with aims of the national programme and was deliberately not influenced by the study design or the research personnel. For ease of interpretation, prescribed training is characterised using the adopted terminology as either "<UT2"; a training intensity below the onset of blood lactate accumulation or ">UT2"; an intensity above the onset of blood lactate accumulation. These are well established for each rower, based on regular and routine physiological testing conducted by sports scientists.

Wellness and Sleep

Subjective assessments of wellbeing and sleep quality were recorded daily using a smartphone application developed by the English Institute of Sport. The app contained 5 questions; ratings of perceived energy level (0-10 scale), perceived "shape" (0-10 scale), perceived freshness (0-10 scale), perceived sleep quality (0-10 scale), and perceived sleep duration (minutes).

Statistical analysis

All statistics were carried out using R software. The distributions of all variables were assessed for normality and the presence of outliers with box-plots. To determine differences between means for healthy rowers and injured and ill rowers independent T-tests were used, with Cohen's d effect sizes (d) used to calculate the magnitude of the standardised difference in means where significant, and reported as 0.2 (small), 0.5 (moderate), 0.8 (large), and 1.3 (very large).

There are two main outcomes for analysis, time to illness and time to injury. We treat both as time-to-event variables, namely that we have both an event (e.g. ill/not ill) and a time of illness (day). The time of illness for those athletes who were never ill is set to the length of the study, i.e. these athletes are said to be censored at the end of the study. Illness and injury are both modelled using a Cox proportional hazards model [2], which is the most commonly used approach for time-to-event variables. For each variable under examination (FORD, FORT and OSI) we estimate a hazard ratio for a 0.5 unit increase in FORD, FORT and OSI, since a 1-unit change is not realistic. A hazard ratio of 1 represents no effect of the variable being tested, below 1 represents a protective effect and above 1 represents a harmful effect. A further complication with these data analysis of these data is that athletes can become ill or get injured multiple times. This repeated measure of illness or injury is correlated over time, and we must account for this correlation (i.e. that an athlete who gets ill may be more likely to get ill again) as well as the correlation between OS biomarkers over time. To account for such repeated measures of OS, illness and injury in this study we included a so-called frailty term in the Cox model for each athlete ID. This term controls for the correlation that is likely present in the outcomes over time. To control for potential confounding effects, we included age and BMI (kg/m^2) in models of both illness and injury.

We use 95% confidence intervals to provide evidence as to whether our sample results are likely to infer population effects for all athletes represented by this sample. We also report p-values, but do not use a stringent cut-off of 0.05 to determine a population effect. We use a strength of evidence approach [19], whereby smaller p-values suggest stronger evidence for a population effect. Data are presented as mean ± SD.

Results

A total of 140 blood samples were drawn in the rowers across the competitive phase. OS testing compliance was very good, at 93%. In contrast, compliance across the training period was poor for the entry of the wellness and sleep data in the smart phone app. All 10 rowers entered data at various time points throughout, however, compliance ranged from 7% to 100% on an individual level, and 44% overall for the squad. Of the ten rowers, eight competed in the World Championships at the end of the monitoring period.

Training load

The prescribed training load across the competition phase, through to the World Championships is presented in Figure 1. The peak in training load occurred in week 13, totaling 227.5 km, including 41 km performed above UT2 intensity.

Figure 1 here

Injury and Illness

All injuries were of rib, back or hip in origin. No additional medical details are included with regards to the illnesses (i.e. infections) and injuries due to issues of confidentiality.

The results in Figure 2 do not account for the correlation of repeated measurements within athletes over time but give a simple comparison of levels of biomarkers across health status. Lower FORD ($p=0.02$), higher FORT ($p=0.063$) and higher OSI ($p=0.003$) were observed on days where an athlete was ill, while higher FORT ($p<0.001$) and OSI ($p=0.017$) were observed in measurements taken while an athlete was injured.

Figure 2

Modelling

The results of a Cox regression of the time to illness and injury are given in Table 3.

Table 1 here

Modelling illness

There was evidence for a protective effect of FORD on illness, with a 0.5 $mmol \cdot L^{-1}$ increase in FORD associated with a 30.6% risk reduction for illness in this sample (hazard ratio 0.694). A 95% confidence interval for this estimate suggests that in a population of endurance athletes, the mean risk reduction is likely between 7% and 48.2% (hazard ratio 95% CI 0.518, 0.93; p=0.014). OSI had a harmful effect on illness risk, with a 0.5 unit increase in OSI being associated with a 11.3% increased risk of illness in this sample (hazard ratio 1.113, 95% CI 1.007, 1.231; p=0.036) on average.

Modelling injury

There was only weak evidence for a harmful effect of OSI, with a 0.5 unit increase associated with a 8.3% increased risk of injury in this sample (hazard ratio 1.083). The 95% confidence interval suggests that this estimate could range from a 1.3% reduction in injury risk to a 19% increased risk (hazard ratio 95% CI 0.987, 1.19; p=0.094).

Haematology and biochemistry

See table 2.

Discussion

The aim of the study was to identify the relationship between injury, illness (i.e. infections) and biomarkers of OS. We report for the first time, strong associations between redox biomarkers and both illness and injury in elite male rowers. OSI was higher in both ill and injured athletes, furthermore it was associated with increased risk of both illness and injury when analysed through a Cox proportional hazards model. Given the low sample size these results merit further investigation in a larger group.

Illness

The higher OSI (lower FORD and higher FORT) observed in the rowers with infections can be explained by the increased production of reactive oxygen and nitrogen species that occurs with activated leukocytes, namely the phagocytic, polymorphonuclear leukocytes, in the presence of a bacterium or virus. We have not reported the types of infection encountered by the rowers (e.g. whether bacterial or viral, or of upper respiratory, gastrointestinal tract in origin) for reasons of confidentiality, however, both viral and bacterial infections can give rise to OS. Evidence of increased OS in physically active individuals with infection and acute and severe infection has previously been reported [20], and a relationship between performance, illness and OS has previously been documented in elite athletes. For example, in a longitudinal study of elite alpine skiers, those athletes classified as "impaired performers" had approximately double the total peroxide load (H_2O_2 equivalents) and were more prone to infection than those skiers classified as "good performers" [9]. The fact that a 0.5 $mmol \cdot L^{-1}$ increase in blood antioxidant capacity (i.e. FORD) in the present study was associated with a ~30% risk reduction in illness, warrants further investigation in other athletic populations. It is noteworthy that others have reported a diminished plasma antioxidant capacity in the overloaded/overtrained athlete [8,21], and overtrained athletes are reported to be more susceptible to illness [22]. Furthermore, in testing the rower's plasma ascorbate (vitamin C) (added to the profile as part of a nutritional screen; see Table 2), a number of the squad were observed to have a marginal vitamin C status (<23 $umol \cdot L^{-1}$) , which may have contributed to low FORD values and reported illness. The ability to track and quantify the athlete's risk of illness, would be of value to any sports science team, given that significant proportions of training time lost to illness does occur. Indeed, UK Sport and the English Institute of Sport estimated that for the 12 months prior to the Rio 2016 Olympics, 108,845 days and 17,173 days of training were lost to injury and illness respectively, as a result of 4685 separate injury or illness incidents, experienced by 1144 athletes across 38 sports (UK Sport, unpublished data). Clearly athletes who are repeatedly ill, and therefore lose training time due to illness, are more likely to experience competition failure [1].

It is of interest, that the doubling of antioxidant foods in elite endurance athletes training at altitude (Sierra Nevada) increases plasma antioxidant capacity and attenuates inflammation without impeding training adaptations (Koivsto et al. 2019). Whilst the administration of encapsulated dried fruit and vegetable concentrates (EDFC) increase plasma antioxidant capacity and circulating gamma T-cells leading to fewer (p=0.07) self-reported total illness symptoms in healthy students (Nantz et al, 2006). Indeed, the same EDFC administered to special forces results in a similar trend, with fewer duty days lost to illness (p=0.06) and a significant decline in inflammation and oxidative stress (Lamprecht et al. 2007). To our knowledge, fruits and vegetables (FV) (dried or whole) have not been shown to attenuate adaptations to exercise. Thus, increasing FV consumption increases plasma antioxidant capacity, and may reduce infection risk,

and such advice should be given to athletes for illness prevention. Whilst speculative, increasing FV whilst ill, may serve as to attenuate symptoms and lead to faster resolution of the infection through moderating oxidative stress and immune responses. Finally, it is noteworthy, that despite the rowers receiving World class performance nutrition support, we observed a marked decline for some antioxidant nutrients (i.e. alpha-carotene, lutein, vitamin C; see table 2) in individual athletes across the period of monitoring; with borderline nutrient status for vitamin C in 2 cases. Despite very limited research in athletes, others have previously reported such findings in elite athletes for carotenoids post altitude training (Pialoux et al.). Although the beyond the scope of this paper, we feel this is an area that requires further research in view of the importance of antioxidant nutrients for the health of the athlete.

Injury

OS biomarkers correlate with training load and volume [7,8], and exceedingly high training loads and/or spikes in training load are a recognised risk factor for injury [2]. Furthermore, OS has been suggested to play a role in the pathophysiology of overuse injuries [23]. We report a significant difference between OSI and FORT biomarkers for injured versus healthy rowers, but not FORD (Figure 2). In modelling the data, there was some evidence for a harmful effect of the OSI with regards to injury, with a 0.5 unit increase associated with an 8.3% increased risk of injury in this sample (hazard ratio: 1.083). In the present study, the rowers were monitored between May and September in the competition phase, a period in which both the training volumes and the potential for injury could be lower than the general preparation training phase in the winter. Indeed, a higher injury rate has been reported in the winter months in international rowers, coinciding with periods of highest training volumes [11]. Had a greater number of injuries resulted, stronger associations with injury and OSI maybe have been evident. Further research is required to explore this relationship in a larger cohort of elite athletes. Moreover, in order to test the validity of monitoring redox biomarkers as a means of guiding strategies aimed at protecting the athlete and mitigating against injury, the monitoring period should encompass the entire season to include the general preparation phase.

Strengths and limitations

FORT and FORD data were repeatedly measured in the same athletes overtime, which allows for stronger inferences to be made compared with a cross-sectional study. We used Cox proportional hazard models to model time-to-event outcomes, while including a frailty term to account for the within player correlation in FORT and FORD. A limitation was the lack of data available on the study athletes, such as profile of mood states, sleep quality and muscle soreness data, which may have provided more information on the relationship between illness/injury and OS.

We have previously highlighted limitations associated with the blood-based OS biomarkers used in this study [17], and others have recently reviewed the topic of redox biomarkers [24]. The FORT (e.g. hydroperoxides) and FORD (e.g. plasma antioxidant capacity) biomarkers provide minimal insight into the complexity of redox signaling and associated cellular changes. The fact that such biomarkers provide little mechanistic insight into cellular redox pathways is of lesser importance in the context of how these biomarkers are applied in the field or the clinic setting. Indeed, the

primary purpose of deploying such biomarkers in the field is to generate real time results (as opposed to a delayed retrospective analysis) to monitor athlete "stress" and recovery, and assist in determining the appropriate hormetic "stimulus" for adaptation in the context of the athletes own historic data. Crucially, these point of care tests are useful to be able to inform athlete management decisions being made at the time of sampling.

It should be recognised that the athletes in this study were part of the British elite sport system and included athletes of the very highest caliber (i.e. Olympic Champion), with all athletes being supported by sports science and medicine practitioners. All athletes were healthy on commencing the study as evidenced in the biomarker wellness screen (Table 2). In addition, the research can be considered to have high ecological validity, as the rowers did not change their routines, diets, or planned training based on any of the monitoring data. In fact, it is clear the rowers engaged and complied with the weekly blood monitoring, which contrasts with the compliance to the subjective wellness data collection; despite entry and collection being simplified through the use of a smartphone application and the questions being tailored to the rowers and their environment.

Practical considerations

OS is increased in injured and ill athletes, and the monitoring of OS may be advantageous assessing recovery from, and in reducing injury and illness risk given the association. In addition, avoiding sustained OS may be advantageous in reducing injury and illness occurrence in individual endurance athletes, however further research is needed. The data presented here provide preliminary evidence that both infection and injury risk can be inferred with measurable changes in OS. Given the inherent practical challenges that come with subjective data collection (e.g. long-term athlete compliance, reliability of the data entered), these biomarkers provide an objective means of quantifying "stress" in the clinic or in the field (e.g. an overseas training camp) for sports science and medicine practitioners.

Conclusions

We have demonstrated that plasma antioxidant capacity (i.e. FORD) may exert a protective effect with regards to the risk of illness, and that OSI is associated with both injury and illness in a cohort of elite rowers. Similar studies in other Olympic and professional sports are needed to corroborate these findings and to prospectively deploy these blood tests to reduce injury and illness.

Acknowledgements. We wish to thank the all athletes and the coaches for agreeing to participate in the research. The results of the current study do not constitute endorsement of the product by the authors or the journal.

Funding. The research was funded by the English Institute of Sport.

Authors contributions

NAL, RB, SM, GT, MH, AR. Conception and design of the study

SM, GT, MH, AR. Data collection

NAL, AJS, CRP, RB. Analysis and interpretation of the work

NAL, AJS, CRP, RB. Drafting of the manuscript

NAL, AJS, CRP, RB, SM, GT, MH, AR. Reviewed and approved the final version of the manuscript.

All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship and are listed.

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Figures 1

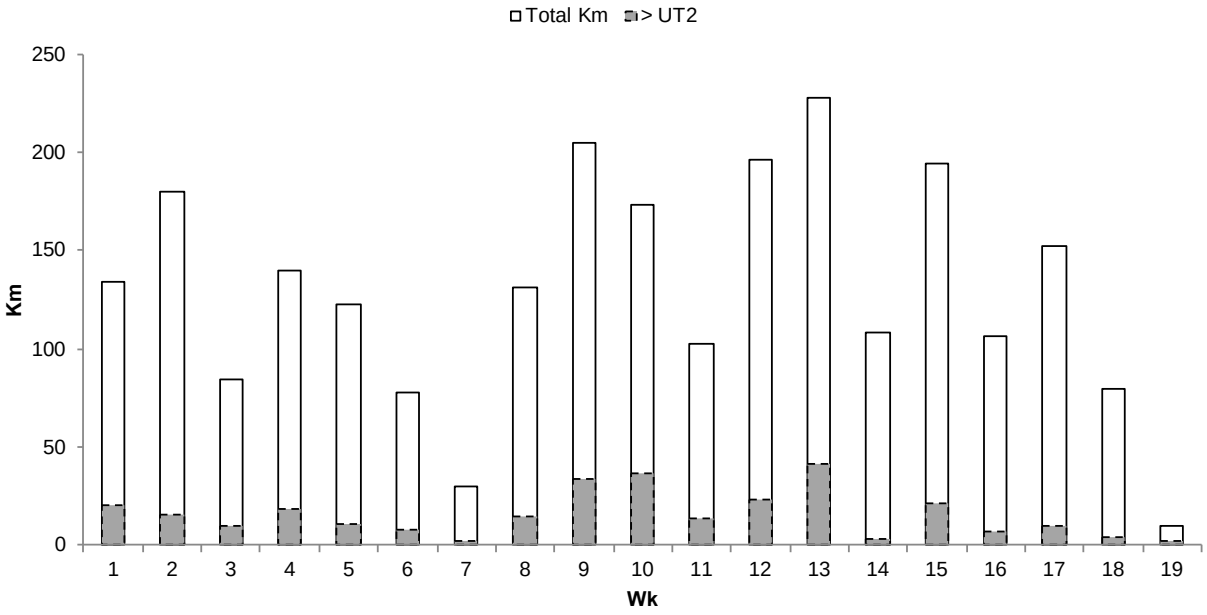
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Figure 2

Figure captions

Figure 1. Weekly training volume measured in kilometres. Bars indicate total weekly training including both on-water rowing and land-based rowing ergometry. Filled in (greyed out) proportions of the bars indicate weekly training volume at an intensity greater than UT2 (above lactate threshold).

Figure 2: FORD, FORT and OSI measurements comparing healthy and ill individuals; and healthy and injured individuals