## Methods

### Study participants

Data were available from 23 American Football athletes participating in Division 1 level in the National Collegiate Athletic Association (NCAA). Repeated measures were collected across training camp and the regular season, spanning 12.5 weeks (88 days) of pre-competitive (2 weeks of summer conditioning, 4 weeks of camp) and competition phases (6.5 weeks). Athletes provided informed consent to participate in data collection throughout the season as part of the athlete support process and the institutional ethics committee at the XXXXX, with YYYYYY providing ethical approval for the research.

### Study design

Longitudinal measures of injury, internal and external load were collected. All contact injuries were recorded by the team’s athletic medical staff, the injury was classified and recorded into an athlete management system categorised by the incident, date, location and mechanism as suggested by previous research. Each injury was reviewed and confirmed by the team’s sports physician. Non-contact injuries were reported to the athletic medicine staff and required withdrawal from athletic participation and were included in the analysis (regardless of ensuing time-lost or not on subsequent days) as this type of injury is considered largely preventable(14, 15, 26). Six key internal load markers were identified: fatigue, sleep quality and muscle soreness from self-reported wellness questionnaires, oxidative stress biomarkers (hydroperoxides; HPx, mmol/L), total anti-oxidant capacity (TAC, mmol/L), and oxidative stress index (OSI; HPx/TAC). Three external training load markers (total distance [m], high intensity distance [m], PlayerloadTM) were also collected. Some indoor sessions took place meaning that distance was not measured on all days. Acute (7-day) and chronic (21-day) PlayerloadTM were calculated using an exponentially weighted moving average (EWMA) method on the total PlayerloadTM variable. The study design involved longitudinal collection of biomarkers (median 10 measures per player), training load (median 46 measures per player) and wellness questionnaires (median 48 measures per player) over 88 days.

### Wellness questionnaires

Over the course of the season the athletes completed a wellness questionnaire(11) two hours before training each day as part of their daily routine. In this questionnaire, players were asked to record perceived feelings of fatigue, sleep quality and overall muscle soreness with a validated(17) 5-point Likert scale (1= very poor, 2 = poor, 3 = average, 4 = good, and 5 = very good). For soreness, players were asked to identify on an anatomical image the location of their sore body part and then rate this soreness level of that specific location on a scale from 1 to 5, with 1 indicating little soreness and 5 being extreme soreness.

### Biomarkers of oxidative stress

A blood sample was taken from each player once per week, with 5-6 players sampled each day. Sampling order of players was randomised each week and samples were collected in the morning before completing the wellness questionnaires and prior to breakfast. Participants arrived at the practice facility in a fasted state, apart from consuming up to 500 mL of water *ad libitum*. Participants were seated, the fingertip cleansed with an alcohol swab and left to dry. The first drop of blood was wiped from the skin with a cotton bud to avoid contamination. 300uL of capillary blood was drawn into a heparinised capillary tube, capped, immediately refrigerated at 5oC and analyzed within 30 minutes. 50uL and 20uL of capillary blood were transferred into separate capillary tubes for TAC (FORD assay, Callegari SpA, Italy) and HPx (FORT assay, Callegari SpA, Italy) analysis respectively, the appropriate reagents were added, inverted several times to mix, centrifuged at 5000 r.min-1 (2000g) for 1 min, and analysed at 37oC with an absorbance wavelength of 505nm using a Callegari CR3000 analyser (Callegari SpA, Italy) according to the manufacturer’s instructions(25).

### Load

Player workloads were determined via accelerometry data(2) calculated from movements in the X, Y and Z axis, expressed as arbitrary units (AU) termed PlayerloadTM via the manufacturer’s software (Catapult Sports, Openfield software, version 1.11.1) for every session. Participants wore the same device, located in the center of the back above the scapulae during every training session and match (2). PlayerloadTM has previously been used to calculate injury risk in collegiate American Football (21, 22) and was chosen as the only variable that could consistently calculate acute and chronic workloads in both indoor and outdoor sessions. Exponentially weighted moving average periods consider the decaying nature of fatigue and fitness and are considered a more appropriate workload measure(49) and were calculated before filtering the main training sessions and games. The acute period was defined as 7-days and the chronic as 21-days in line with previous American Football research(33, 34).

**References**

1. **Bahr R, and Holme I**. Risk factors for sports injuries—a methodological approach. *Br J Sports Med* 37: 384-392, 2003.

2. **Boyd LJ, Ball K, and Aughey RJ**. The reliability of MinimaxX accelerometers for measuring physical activity in Australian football. *Int J Sports Physiol Perform* 6: 311-321, 2011.

3. **Bredt SdGT, Chagas MH, Peixoto GH, Menzel HJ, and de Andrade AGP**. Understanding player load: meanings and limitations. *J Hum Kinet* 71: 5-9, 2020.

4. **Carey DL, Ong K, Whiteley R, Crossley KM, Crow J, and Morris ME**. Predictive modelling of training loads and injury in Australian football. *Int. J. Comput. Sci. Sport.* 17: 49-66, 2018.

5. **Cox DR**. Regression models and life tables. *J R Stat Soc Series B Stat Methodol* 34: 187-220, 1972.

6. **DePhillipo NN, Aman ZS, Kennedy MI, Begley J, Moatshe G, and LaPrade RF**. Efficacy of vitamin C supplementation on collagen synthesis and oxidative stress after musculoskeletal injuries: a systematic review. *Orthop. J. Sports Med.* 6: 2325967118804544, 2018.

7. **Drew MK, and Finch CF**. The relationship between training load and injury, illness and soreness: a systematic and literature review. *Sports Med* 46: 861-883, 2016.

8. **Dunnill C, Patton T, Brennan J, Barrett J, Dryden M, Cooke J, Leaper D, and Georgopoulos NT**. Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS‐modulating technologies for augmentation of the healing process. *Int. Wound J.* 14: 89-96, 2017.

9. **Finch C**. A new framework for research leading to sports injury prevention. *J Sci Med Sport* 9: 3-9, 2006.

10. **Finch CF, and Cook J**. Categorising sports injuries in epidemiological studies: the subsequent injury categorisation (SIC) model to address multiple, recurrent and exacerbation of injuries. *Br J Sports Med* 48: 1276-1280, 2014.

11. **Fullagar HH, Govus A, Hanisch J, and Murray A**. The time course of perceptual recovery markers after match play in Division IA college American football. *Int J Sports Physiol Perform* 12: 1264-1266, 2017.

12. **Fullagar HH, McCunn R, and Murray A**. Updated review of the applied physiology of American college football: physical demands, strength and conditioning, nutrition, and injury characteristics of America’s favorite game. *Int J Sports Physiol Perform* 12: 1396-1403, 2017.

13. **Fuller CW, Molloy MG, Bagate C, Bahr R, Brooks JH, Donson H, Kemp SP, McCrory P, McIntosh AS, and Meeuwisse WH**. Consensus statement on injury definitions and data collection procedures for studies of injuries in rugby union. *Br J Sports Med* 41: 328-331, 2007.

14. **Gabbett TJ**. The development and application of an injury prediction model for noncontact, soft-tissue injuries in elite collision sport athletes. *J. Strength Cond. Res.* 24: 2593-2603, 2010.

15. **Gabbett TJ**. The training—injury prevention paradox: should athletes be training smarter and harder? *Br J Sports Med* 50: 273-280, 2016.

16. **Gabbett TJ, Ullah S, and Finch CF**. Identifying risk factors for contact injury in professional rugby league players–application of a frailty model for recurrent injury. *J Sci Med Sport* 15: 496-504, 2012.

17. **Gastin PB, Meyer D, and Robinson D**. Perceptions of wellness to monitor adaptive responses to training and competition in elite Australian football. *J. Strength Cond. Res.* 27: 2518-2526, 2013.

18. **Govus AD, Coutts A, Duffield R, Murray A, and Fullagar H**. Relationship between pretraining subjective wellness measures, player load, and rating-of-perceived-exertion training load in American college football. *Int J Sports Physiol Perform* 13: 95-101, 2018.

19. **Hewett TE**. Prediction of future injury in sport: primary and secondary anterior cruciate ligament injury risk and return to sport as a model. *J Orthop Sports Phys Ther* 47: 228-231, 2017.

20. **Hewett TE, Webster KE, and Hurd WJ**. Systematic selection of key logistic regression variables for risk prediction analyses: a five factor maximum model. *Clin J Sport Med* 29: 78, 2019.

21. **Hulin BT, Gabbett TJ, Lawson DW, Caputi P, and Sampson JA**. The acute: chronic workload ratio predicts injury: high chronic workload may decrease injury risk in elite rugby league players. *Br J Sports Med* 50: 231-236, 2016.

22. **Impellizzeri FM, Marcora S, Castagna C, Reilly T, Sassi A, Iaia F, and Rampinini E**. Physiological and performance effects of generic versus specific aerobic training in soccer players. *Int J Sports Med* 27: 483-492, 2006.

23. **Laird NM, and Ware JH**. Random-effects models for longitudinal data. *Biometrics* 963-974, 1982.

24. **Lathlean TJ, Gastin PB, Newstead SV, and Finch CF**. Player wellness (soreness and stress) and injury in elite junior Australian football players over 1 season. *Int J Sports Physiol Perform* 15: 1422-1429, 2020.

25. **Lewis NA, Newell J, Burden R, Howatson G, and Pedlar CR**. Critical difference and biological variation in biomarkers of oxidative stress and nutritional status in athletes. *PLoS One* 11: e0149927, 2016.

26. **McCunn R, Fullagar HH, Williams S, Halseth TJ, Sampson JA, and Murray A**. Playing experience and position influence injury risk among NCAA Division I collegiate footballers. *Int J Sports Physiol Perform* 12: 1297-1304, 2017.

27. **Meeuwisse WH, Tyreman H, Hagel B, and Emery C**. A dynamic model of etiology in sport injury: the recursive nature of risk and causation. *Clin J Sport Med* 17: 215-219, 2007.

28. **Nielsen RO, Bertelsen ML, Ramskov D, Møller M, Hulme A, Theisen D, Finch CF, Fortington LV, Mansournia MA, and Parner ET**. Time-to-event analysis for sports injury research part 1: time-varying exposures. *Br J Sports Med* 53: 61-68, 2019.

29. **Nielsen RO, Bertelsen ML, Ramskov D, Møller M, Hulme A, Theisen D, Finch CF, Fortington LV, Mansournia MA, and Parner ET**. Time-to-event analysis for sports injury research part 2: time-varying outcomes. *Br J Sports Med* 53: 70-78, 2019.

30. **R Development Core Team**. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria, [http://www.r-project.org/](http://www.R-project.org), 2012.

31. **Rizopoulos D, Verbeke G, and Molenberghs G**. Shared parameter models under random effects misspecification. *Biometrika* 95: 63-74, 2008.

32. **Rossi A, Pappalardo L, Cintia P, Iaia M, Fernandez J, and Medina D**. Effective injury prediction in professional soccer with GPS data and machine learning. *arXiv preprint arXiv:170508079* 2017.

33. **Sampson JA, Murray A, Williams S, Halseth T, Hanisch J, Golden G, and Fullagar H**. Injury risk-workload associations in NCAA American college football. *J Sci Med Sport* 21: 1215-1220, 2018.

34. **Sampson JA, Murray A, Williams S, Sullivan A, and Fullagar HH**. Subjective wellness, acute: chronic workloads, and injury risk in college football. *J Strength Cond Res* 2019.

35. **Sterne JA, and Smith GD**. Sifting the evidence—what's wrong with significance tests? *Phys Ther* 81: 1464-1469, 2001.

36. **Thorpe RT, Atkinson G, Drust B, and Gregson W**. Monitoring fatigue status in elite team-sport athletes: implications for practice. *Int J Sports Physiol Perform* 12: S2-27-S22-34, 2017.

37. **Toohey LA, Drew MK, Fortington LV, Menaspa MJ, Finch CF, and Cook JL**. Comparison of subsequent injury categorisation (sic) models and their application in a sporting population. *Inj. Epidemiol.* 6: 9, 2019.

38. **Van Mechelen W, Hlobil H, and Kemper HC**. Incidence, severity, aetiology and prevention of sports injuries. *Sports Med* 14: 82-99, 1992.

39. **Vanrenterghem J, Nedergaard NJ, Robinson MA, and Drust B**. Training load monitoring in team sports: a novel framework separating physiological and biomechanical load-adaptation pathways. *Sports Med* 47: 2135-2142, 2017.

40. **Vaupel JW, Manton KG, and Stallard E**. The impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography* 16: 439-454, 1979.

41. **Venturelli M, Schena F, Zanolla L, and Bishop D**. Injury risk factors in young soccer players detected by a multivariate survival model. *J Sci Med Sport* 14: 293-298, 2011.

42. **Weaving D, Whitehead S, Till K, and Jones B**. Validity of real-time data generated by a wearable microtechnology device. *J. Strength Cond. Res.* 31: 2876-2879, 2017.

43. **Wellman AD, Coad SC, Flynn PJ, Siam TK, and McLellan CP**. Perceived wellness associated with practice and competition in National Collegiate Athletic Association Division I football players. *J. Strength Cond. Res.* 33: 112-124, 2019.

44. **Wellman AD, Coad SC, Goulet GC, and McLellan CP**. Quantification of accelerometer derived impacts associated with competitive games in National Collegiate Athletic Association Division I college football players. *J. Strength Cond. Res.* 31: 330-338, 2017.

45. **Whalan M, Lovell R, and Sampson JA**. Do niggles matter?-increased injury risk following physical complaints in football (soccer). *Sci Med Football* 4: 216-224, 2020.

46. **Wilkerson GB, and Colston MA**. A refined prediction model for core and lower extremity sprains and strains among collegiate football players. *J. Athl. Train.* 50: 643-650, 2015.

47. **Wilkerson GB, Gupta A, Allen JR, Keith CM, and Colston MA**. Utilization of practice session average inertial load to quantify college football injury risk. *J Strength Cond Res* 30: 2369-2374, 2016.

48. **Williams S, Booton T, Watson M, Rowland D, and Altini M**. Heart rate variability is a moderating factor in the workload-injury relationship of competitive crossfit™ athletes. *J Sports Sci Med* 16: 443, 2017.

49. **Williams S, West S, Cross MJ, and Stokes KA**. Better way to determine the acute: chronic workload ratio? *Br J Sports Med* 51: 209-210, 2017.

50. **Windt J, and Gabbett TJ**. How do training and competition workloads relate to injury? The workload—injury aetiology model. *Br J Sports Med* 51: 428-435, 2017.

51. **Wood S**. Generalized Additive Models: An Introduction with R.,(Chapman and Hall: CRC Press, Boca Raton, FL.). 2006.

52. **Wood SN**. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J R Stat Soc Series B Stat Methodol* 73: 3-36, 2011.

53. **Wood SN**. *Generalized additive models: an introduction with R*. Chapman and Hall/CRC, 2017.

54. **Xu C, Baines PD, and Wang J-L**. Standard error estimation using the EM algorithm for the joint modeling of survival and longitudinal data. *Biostatistics* 15: 731-744, 2014.