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Håvard Wiig

Physiological and perceived exertion responses to training and match load in football

External and internal load, neuromuscular fatigue, muscle damage, and recovery

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Summary

A tight match schedule in elite football makes it challenging to balance training and match load with recovery and rest. Being able to reliably measure the external load and understand how a given amount of load affects the experience of internal load, neuromuscular fatigue, recovery time and physical performance for the individual player may be the key to balancing this as best as possible. In this thesis we explore the association between external load, measured with player tracking devices and internal load (perceived effort throughout the training session) and individual differences in this context (Paper I). Furthermore, we investigate how external load in a football match affects the subsequent changes in blood markers for muscle damage, and the recovery of neuromuscular function and physical performance (Paper II). Finally, we explore to which extent a football match leads to ultrastructural damage to the muscle fibers, via Heat Shock Proteins (HSP) as proxy markers, and how this relates to fatigue and recovery of muscle function (Paper III).

A total of 99 football players participated in two different studies. In study I we followed the same players over several training sessions in a 32-week period and measured external load with player tracking devices and internal load with the *session rating of perceived exertion-derived training load* method (sRPE-TL; Paper I). In study II, we measured external load in three matches, one match per player, and followed the subsequent recovery process with measurements of creatine kinase (CK), myoglobin, countermovement jump (CMJ), 30 m sprint and YO-YO intermittent recovery test (Paper II), and the stress response in muscle fibers with analyses of HSP in muscle biopsies from m. vastus lateralis (Paper III).

The results from study I showed that the difference between training sessions with typical low and high external load (2 standard deviations of the variable PlayerLoad^{TM}), led to a 106 % (90 % confidence interval; CI; 83–133 %, effect size; ES; of 2.52–2.68) increase in sRPE-TL (*within-player* effect), with an individual response of ±24 % (CI; 10–33, ES = 0.76). Furthermore, we found a difference of 19% (CI; 3–38, ES = 0.64) between players with low versus high average PlayerLoad^{TM} (*between-player* effect). Finally, we observed that the variation in sRPE-TL from session to session was 21% (CI; 13–27, ES = 0.68) after adjustment for PlayerLoad^{TM} and individual differences in sRPE-TL.

The results from study II showed a reduction in CMJ-performance, and increases in CK and myoglobin with effect sizes of −0.75, 0.92 and 3.80 respectively 1 h after the match. Of the external load variables, high speed running distance, had a consistent effect on changes in CK, 1–72 h after the match (ES = 0.60–1.08). Total distance had a small effect (ES = 0.56) on the 30 m sprint 72 h after the match. The effect of the investigated external load

variables on CMJ performance were either trivial or unclear, even though CMJ height was the performance indicator with the most consistent reductions in the recovery period.

In the subgroup of players that donated muscle biopsies, we observed a decrease in soluble HSPs (the cytosol fraction) of 15–17 % ($p < 0.01$) 1 h after the match. Concurrently, HSPs bound in the cytoskeletal fraction increased 3.6 and 1.8 times the baseline levels ($p < 0.01$). For αB-Crystallin, which is a small HSP rapidly binding to denatured proteins, the increased levels bound in the cytoskeletal fraction returned to baseline levels after 72 h, whereas HSP70, which is a larger HSP involved in the repair process, remained elevated. With immunohistochemistry methods on frozen muscle cross section, we found a 20–27 % increase in staining intensity for the two HSPs in myofibrillar structures ($p < 0.01$) 1 h after the match in both Type I and Type II fibers. Staining intensity did not return to baseline level within 72 h. In addition, there was a 2.2-fold increase in the proportions of fibers that showed granular staining patterns of αB-Crystallin, indicating sarcomere disruption, 1 hour after match ($p < 0.01$).

In summary, in study I there was a close relationship between external load, measured by player tracking devices, and internal load measured with the sRPE-TL method, where external load variables with a low or no intensity-threshold showed the strongest relationship with sRPE-TL. This confirms that measurements of external load with player tracking devices is a valid method monitoring training load, but also that some external load variables are better than others. Nonetheless, we observed large individual differences in the effect of external load on internal load, which emphasizes the necessity of individual follow-up as a fixed quantity of external load leads to different perceived exertion for a set of players. Lastly, we observed large variation in internal load between sessions that could not be explained by the external load variables or the individual response to them. This suggest that there are loading patterns that are not captured by the external load variables. Based on these results, we recommended to use both measurements of the internal and external load, where the importance of individual follow-up is emphasized.

Furthermore, the results in study II showed that the amount of high speed running distance in a match was positively associated with increased levels of the blood markers of muscle damage both immediately and 72 h after match. Total distance and PlayerLoad^{TM} had a negative effect on 30 m sprint performance 72 h after match, whereas surprisingly no relationship was found between the measured external load variables and CMJ, even though CMJ performance was strongly reduced after the match. The results suggest that several different external load variables should be chosen for the evaluation of match load as these can provide different information about the recovery period. Although external load variables showed an effect on time to recovery at the group level, there was not enough statistical power to predict the recovery outcome of the individual player.

Finally, we found that the HSP stress response in muscle fibers, increased levels of blood markers for muscle damage, decreased neuromuscular function

and increased perceived muscle soreness indicates *mild* muscle damage after football match. Such ultrastructural muscle damage likely plays a role in the prolonged recovery time after match. Compared to studies where the load on the muscle is unfamiliar or extreme, football matches resulted in considerably lower HSP response. This means that the players are generally well-adapted to the match load, but there are still loading patterns that exceeds the tolerability threshold and results in muscle damage, hence a subsequent slow recovery of muscle function.

Sammendrag

Tett kampprogram i toppfotball gjør det utfordrende å balansere trening- og kampbelastning med restitusjon og hvile. Det å pålitelig kunne måle den ytre belastningen og forstå hvordan en gitt mengde belastning påvirker opplevelsen av indre belastning, nevromuskulær tretthet, restitusjonstid og fysisk prestasjon for enkeltspilleren kan være nøkkelen til å best mulig balansere dette. I denne avhandlingen utforskes sammenhengen mellom ytre belastning, målt med bevegelsessensorer, og indre belastning, målt som opplevd anstrengelse i treningsøkten, og om det er individuelle forskjeller i denne sammenhengen (artikkel I). Videre utforskes hvordan ytre belastning i en fotballkamp påvirker den påfølgende restitusjonen av blodmarkører for muskelskade, nevromuskulær funksjon og fysisk prestasjon (artikkel II). Til sist ser vi nærmere på i hvilken grad en fotballkamp fører til ultrastrukturelle skader på muskelfibrene, indikert med akkumulering av Heat Shock Proteiner, på affiserte områder, og om slike skader kan forklare den langsomme restitusjonen etter fotball kamp (artikkel III).

Totalt deltok 99 fotballspillere i to ulike studier. I studie I fulgte vi de samme spillerne over flere treningsøkter og målte ytre belastning med bevegelsessensorer og avledede belastningsvariabler som total distanse, PlayerLoad^{TM} og høyintensitets aksjoner, og den indre belastning med *session rating of perceived exertion-derived training load* metoden (sRPE-TL; artikke I). I studie II målte vi ytre belastning med bevegelsessensorer i en enkelt kamp, for flere lag, og den påfølgende restitusjonsprosessen (1–72 timer) med målinger av kreatinkinase (CK), myoglobin, svikthopp (CMJ), 30 m sprint og YOYO IR 1 test (YOYO; artikkel II) og Heat Shock Proteiner (HSP) fra muskelbiopsier av den laterale brede lårmuskelen (artikkel III).

Resultatene fra studie I viste at differansen mellom økter med lav og høy ytre belastning (tilsvarende 2 standardavvik av belastningsvariablen PlayerLoad^{TM}), førte til 106 % (90 % CI; 83–133 %, ES = 2.52–2.68) økning av sRPE-TL (*innad-i-spiller effekt*), med en individuell variasjon i responsen på ±24 % (CI; 10–33 %, ES = 0.76). Videre fant vi en differanse på 19 % (CI; 83–133 %, ES = 0.64) i sRPE-TL mellom spillere med lav kontra høy gjennomsnittlig PlayerLoad^{TM}. Til sist observerte vi at variasjonen i sRPE-TL fra økt til økt var på 21 % (CI; 13–27 %, ES = 0.68) etter justering for PlayerLoad^{TM} og individuelle forskjeller i sRPE-TL.

Resultatene fra studie II viste en reduksjon i spenst målt i CMJ og en økning i blodmarkørene for muskelskade (CK og myoglobin) med effektstørrelser på henholdsvis −0.75, 0.92 og 3.80, 1 time etter kampen. Av belastningsvariablene så hadde løpsdistanse med høy hastighet en vedvarende effekt på CK 1–72 timer etter kampen (ES = 0.60–1.08). Total distanse hadde en liten effekt (ES = 0.56)

på 30 m sprint 72 timer etter kamp. Effekten av de målte belastningsvariablene på reduksjonen i CMJ var enten trivielle eller uklare.

I undergruppen av spillere som donerte muskelbiopsier observerte vi en reduksjon i de løselige HSP (cytosolfraksjonen) på 15–17 % ($p < 0.01$) 1 time etter kamp. Samtidig så vi en økning i HSP bundet i cytoskjelettfraksjonen på 3.6 og 1.8 ganger hvilenivå ($p < 0.01$). For αB-Crystallin, som er et lite HSP som raskt binder seg til ødelagte proteiner, observerte vi at mengden bundet i cytoskjelettfraksjonen returnerte til utgangsnivåer etter 72 timer, men HSP70, som er et større HSP og mer involvert i reparasjonsprosesser, forble forhøyet. Med en immunohistokjemisk metode som påviser proteiner på tynne snitt av muskelbiopsiene, fant vi en økning i bundet HSP i myofibrillære strukturer på 20–27 % ($p < 0.01$) 1 time etter kamp i både Type I og Type II fibrene. Mengden bundet HSP i myofibrillære strukturer var fortsatt forhøyet 72 timer etter kamp. I tillegg ble det ble det observert en 2.2 ganger økning i andel fiber som viste granularisering av αB-Crystallin, en indikasjon på skade i sarkomerstruktur, 1 time etter kamp ($p < 0.01$).

Oppsummert viser studie I at det er nær sammenheng mellom ytre belastning, målt med bevegelsessensorer, og indre belastning målt med sRPE-TL metoden, hvor belastningsvariabler med en lav eller ingen intensitets-grense hadde sterkest sammenheng med selvopplevd belastning. Dette bekrefter at måling av ekstern belastning med bevegelsessensorer er en valid metode for å monitorere belastning over tid, men viser også at noen belastningsvariabler er bedre enn andre. Vi så derimot store individuelle forskjeller i effekten av ytre belastning på indre belastning, som understreker viktigheten av individuell oppfølging da en lik mengde ekstern belastning ikke medfører en lik opplevd belastning hos spillerne. Til sist fant vi store variasjoner i indre belastning *mellom* treningsøkter som ikke kunne forklares med belastningen målt med bevegelsessensorene, eller de individuelle forskjellene i indre belastning. Dette tyder på at det er belastning i øktene som bevegelsessensorer ikke fanger opp. Ut i fra disse resultatene anbefales det å benytte både måling av indre og ytre belastningen for å monitorere belastning over tid, der viktigheten av individuell oppfølgning understrekes.

Resultatene fra studie II viser at distanse tilbakelagt med høy løpehastighet i kamp har en effekt på muskelskademarkørene målt i blodet både rett etter og 72 timer etter kamp. Total distanse og PlayerLoad^{TM} hadde en negativ effekt på prestasjon på 30 m sprint 72 timer etter kamp, mens vi noe overraskende ikke fant en sammenheng mellom belastningsvariablene og spenst, selv om spensten var kraftig redusert etter kamp. Resultatene antyder at man bør måle flere ulike belastningsvariabler da disse kan gi ulik informasjon om restitusjonstiden. Selv om vi fant en effekt av belastningsvariablene på restitusjonen på gruppenivå, så var ikke dataene sterke nok til å predikere restitusjonsutfallet til enkeltspillere. Til sist fant vi at stressresponsen i muskelfibrene målt med HSP akkumulering, økning i blodmarkørene for muskelskade, nedgang i nevromuskulær funksjon og økning i opplevd muskelsårhet indikerer *milde* muskelskader etter fotballkamp. Slike ultrastrukturelle skader spiller sannsynligvis en rolle i den forlengede restitusjonstiden etter kamp. Sammenliknet med studier hvor belastningen er uvant eller ekstrem og gir store muskelskader, så gav fotballkampene en

betydeliglavere HSP-respons. Det betyr at spillerne generelt sett er tilpasset belastningen fra kamp, men likevel at det er belastningsmønstre som overgår tålegrensen og fører til muskelskade og derav påfølgende langsom restitusjon av muskelfunksjon.

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Porsgrunn, Desember 2023, Håvard Wiig

List of Papers

Paper I

Håvard Wiig, Thor Einar Andersen, Live S. Luteberget and Matt Spencer. (2020). "Individual Response to External Training Load in Elite Football Players". *International Journal of Sports Physiology and Performance*. Vol. 15, no. 5, pp. 696–704. DOI: 10.1123/ijspp.2019-0453.

Paper II

Håvard Wiig, Truls Raastad, Live S. Luteberget, Ingvar Ims and Matt Spencer. (2019). "External Load Variables Affect Recovery Markers up to 72 h After Semiprofessional Football Matches". *Frontiers in Physiology* Vol. 10, DOI: 10.3389/fphys.2019.00689.

Paper III

Håvard Wiig, Kristoffer T. Cumming, Vilde Handegaard, Jostein Stabell, Matt Spencer and Truls Raastad. (2022). "Muscular heat shock protein response and muscle damage after semi-professional football match". *Scandinavian Journal of Medicine and Science in Sports*. Vol. 32, no. 6, pp. 984–996. DOI: 10.1111/sms.14148.

Abbreviations

- AU = Arbitrary Units
- BSA = Bovine Serum Albumin
- CI = Confidence Interval
- CK = Creatine Kinase
- CMJ = Countermovement Jump
- CV = Coefficient of Variation
- ECM = Extracellular matrix
- ES = Effect Size
- GNSS = Global Navigation Satellite System
- HIE = High Intensity Events
- HSP = Heat Shock Protein
- HSRD = High Speed Running Distance ($>4\,\mathrm{m\,s^{-1}}$)[1]
- IMU = Inertial Measurements Units
- LPS = Local Position System
- MBI = Magnitude Based Inferences
- PBS = Phosphate-buffered Saline
- RPE = Rating of Perceived Exertion
- VHSRD = Very-high Speed Running Distance ($>5.5\,\mathrm{m\,s^{-1}}$)
- SD = Standard Deviation
- SP30 = 30 m sprint
- sRPE = Session Rating of Perceived Exertion
- sRPE-TL = Session Rating of Perceived Exertion Training Load (sRPE × Session duration)
- TBS = Tris-buffered Saline
- TRIMP = Heart-rate derived Training Impulse
- YOYO = Yo-Yo Intermittent Recovery Test Level 1
- YOYOmax = The maximal restult of pre- and post-match YOYO tests

[1]The term High Intensity Running (HIR) was used in Paper II

List of Figures

List of Tables

Contents

Chapter 1

Introduction

Professional football teams play a high number of matches over a season, including friendly-, league-, national cup and some international cup matches. As an example, in the 22/23-season, Manchester City FC played 61 official matches, whereof 29 were played only 72 h after the previous match. Therefore, a challenge is to balance the high physical demands playing matches with enough time to recover. Also, between matches, players need to put additional training sessions to increase or maintain their physical fitness to optimize performance in the next match or for the remaining season and furthermore to reduce the risk of injuries. Moreover, periods with fixture congestions with only two to three days between matches, often accompanied with traveling, late-evening matches and high psychological pressure, are highly challenging. To complicate things even further, players may differ in their individual characteristics, training history, playing position, physical fitness, response to training, match minutes, training load and playing style etc. Hence, how can we best manage the team performance while ensuring that each player is managed individually?

An obvious solution would be to quantify, not only the number of trainings and matches, but also the amount of work or *load* that each player completes within each session and match. Furthermore, monitoring fatigue, recovery parameters, response to training and the players' well-being may add additional value. The next step would be to organize the measurements in time, for each player, and then summarize the previous training period and plan for and manage the next period. But what is the correct amount of training load for each player? And how much time is needed to recover between sessions and matches? And how do we measure that?

First, we need valid and reliable measurements tools to quantify training and match load. Global navigation satellite systems (GNSS) and accelerometer technologies are commonly used in high-level football (Akenhead & Nassis, 2016), making it possible to quantify on-field player movements. With parameters such as distance covered, running speed, accelerations and decelerations, they describe the *external load*. However, from the hundreds of different variables and metrics that can be exported from these tracking systems, which should we use? Are these systems able to capture the complex movements, variation in intensity, and durations or repetitiveness of actions that results in the *true* amount of work or load that represent football play?

Let say we do have valid and reliable measures of external load. Will the same amount of external load then impact two different players in a similar manner? The physiological response to the external load can be described as the *internal load*, and it is affected by individual characteristics, training status and environmental factors (Impellizzeri et al., 2019). Examples of measures of

internal load are perception of effort, heart rate, or the oxygen consumption. What is the relationship between the different external load variables and the internal load? How much of the variation in internal load is explained by external load and do players respond differently or the same?

To manage the training load, only measuring external and internal load is not sufficient. We also need to know how fast the players recover after training sessions and matches to know when to schedule the next session and what the content and intensity should be. For example, a football match is physically demanding and produces neuromuscular fatigue, i.e. a reduction in the muscle's ability to exert force that takes 2–4 days to recover from (Nédélec et al., 2012; Silva et al., 2018). That is a problem when the next match is played only 3 days after. Is the time to recovery the same for all players, or is the recovery time dependent on the amount of external load in the match and can we predict the time to recovery from external load? Yet, another question is why the recovery time is so long given that they play matches on a regular basis? A common perception is that the neuromuscular fatigue is partly caused by muscle damage (Nédélec et al., 2012). But what is the evidence for that? And do we find actual damage to the muscle cells?

These questions formed the foundation for the work leading to this thesis. In summary, this thesis aims to address the relationships between internal load and external load, and whether there are individual differences. Furthermore, we wanted to study the relationships between external load variables and recovery after a match. Finally, we investigated to what extend a football match produces damage to muscle cells.

Chapter 2

Background

2.1 Training- and match load in football

To understand what training and match load in football means, it is useful to place the terms in a bigger framework. The following definitions are in line with The International Olympic Committee consensus statement in load in sport and risk of injury (Soligard et al., 2016). Firstly, *load* can be defined as "the sport and non-sport burden (single or multiple physiological, psychological or mechanical stressors) as a stimulus that is applied to a human biological system (including subcellular elements, a single cell, tissues, one or multiple organ systems or the individual)". Load can be applied to the individual human biological system over varying time periods (seconds, minutes, hours to days, weeks, months and years) and with varying magnitude (i.e. duration, frequency and intensity), and a systematic application of load that improves the performance in a specific task is what we generally regard as *training*. After an amount of load is applied, the full return of the biological system to homeostasis is called *recovery*. If there is a positive change in the biological system in response to loading and adequate recovery, we call it *adaptation*, whereas a negative change in response to load with inadequate recovery is called *maladaption*. While continually adaption over time is desirable, congested competition and trainings sessions may lead to *excessive loading* in shorter periods, i.e. loading cycles (including physiological, psychological, travel load and other) with inadequate recovery or rest that manifests as maladaptation, injury, or illness. To reduce the risk of excessive loading, appropriate prescription, monitoring, and adjustment of load, i.e. *load management*, is necessary. *Training load* is "the cumulative amount of stress placed on an individual from a single or multiple training sessions (structured or unstructured) over a period of time" (Soligard et al., 2016). It is useful when monitoring and managing training load to consider it as cumulative over time, because training sessions in sequence, and the recovery process in between, interact. However, in this thesis the focus will be on *load* from single training sessions. Likewise, *match load* is considered as the *load* from a single match.

2.2 Excessive load and injuries in football

Team sports, contrary to many individual sports, are more bound to a competition schedule characterized by a long competitive season with frequent matches, and since the results aggregate, each competition is equally important. That means that every match requires near maximal effort, but also that the time to recover is bound to the time period to the next match. If physical fitness should be developed or maintained, or preparations for the next match be made, it must

be done in the same short window between matches. Furthermore, football is a team sport and many of these training-sessions will be team-sessions and not necessarily optimized for the individual player. When there are many matches or trainings in a short period of time, it may be challenging to balance high training- and match load with enough recovery. Such periods are not necessarily associated with reduced physical performance (Dupont et al., 2010), but risk of injuries is increased (Drew & Finch, 2016; Dupont et al., 2010). Injuries are a major problem in football with incidence of 6.6 injuries per 1000 h, distributed on 23.8/1000 h in matches and 3.4/1000 h in training (Ekstrand et al., 2021). Muscle injuries, mainly hamstrings, adductors, quadriceps and calf muscles, are the most common injuries and account for one third of time loss injures, and a player can expect 0.6 muscle injuries per season (Ekstrand et al., 2011). Hamstrings injuries are most commonly due to running or sprinting and are more likely to occur in the last 15 min of each match halves (Ekstrand et al., 2023). Although we have seen a decrease in overall injury incidence by 3 % per year from 2001 to 2019 in training and match, mainly due to decrease in ligament injuries, muscle injuries have remained constant (Ekstrand et al., 2021). However, there is also evidence that muscle injuries are increasing lately, for example, the last eight years hamstring injury incidence has increased annually by 6.7% and 3.9% for training and matches respectively in European elite clubs (Ekstrand et al., 2023). The authors hypothesizes that more high-intensity activities in matches and crowded calendars with increasing number of matches and travels and less pre-season training sessions could be the cause. Injuries are not only negatively impacting the injured player itself, Hägglund et al. (2013) found that both lower injury burden (days lost per 1000 h) and higher match availability were associated with higher final league ranking and higher points per match. The high number of matches together with the high number of muscle injuries we observe in elite football calls for better injury prevention and load management. Key to this process are good measurement methods for tranining- and match load.

2.3 Measuring load and the distinction between internal and external load

Monitoring and managing training load may assist to reduce injury risk (Gabbett, 2016; McCall et al., 2018) and achieve the desired training outcome (Bourdon et al., 2017). However, monitoring and managing the *true* load is dependent on methods to quantify load accurately and reliably, which is challenging in team sports due to the complexity of movements and actions, and the constant shifting intensities. When quantifying training load, a typical distinction is made between *internal load* and *external load* depending on whether the measurable aspects are occurring internally or externally to the athlete. External load is defined as the work completed by an athlete measured independently of his or her internal characteristics, whereas the internal load is defined as the relative physiological stress imposed on the athlete (Wallace et al., 2009). Hence,

the internal load is determined by an interaction of the external load and the individual characteristics, training status, psychological status, health, nutrition, environment, whether conditions, and genetics of the athlete (Impellizzeri et al., 2019). The relationship between internal and external load is depicted in figure 2.1. Given that it is the stress response and the subsequent adaptations, that determines the training outcome, internal load should be the primary measure when monitoring athletes (Impellizzeri et al., 2019). However, it is difficult to estimate internal load prior to exercise, especially in self-pacing sports such as football, and therefore to prescribe training load to a session. On the other hand, external load is both easy to measure and to prescribe. Hence, a combination of internal and external load measures could be useful in practice to get a more complete picture of the training load, and to monitor and manage training load.

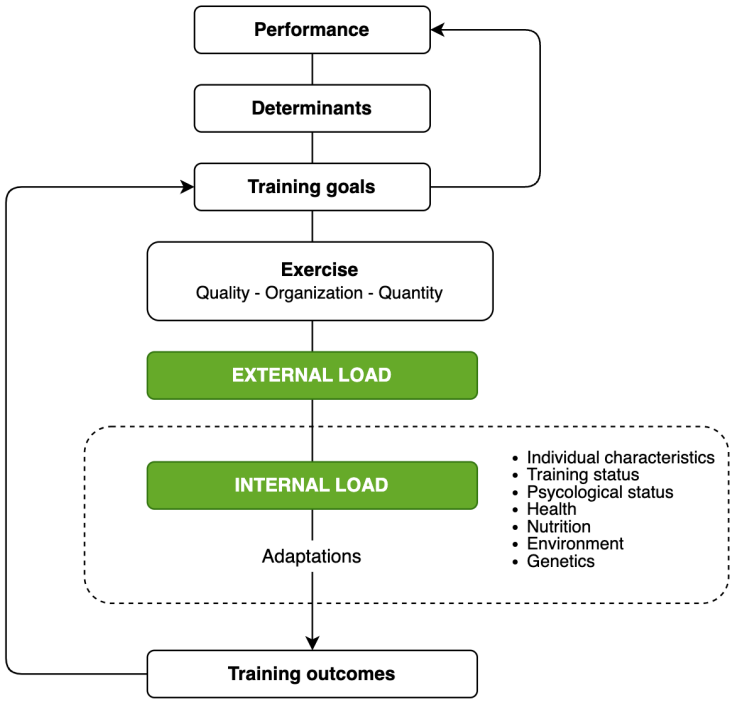


Figure 2.1: A theoretical framework of the training process. Internal training load is determined by individual characteristics, training status, environment etc. and the quality and quantity and organization of external load. Figure is recreated from Impellizzeri et al., 2019.

Although we want to measure the *load* (as defined in section 2.1), no gold standard exists for measuring load in sports due to the very different nature of sports. A measure must be chosen specifically for each activity and the validity of the measure is dependent on the context. For example, internal load is commonly quantified by heart rate (HR) in steady state endurance activity. On the other hand, HR is not valid for resistance training where the amount of external resistance lifted is more appropriate. In sports interspersed with

anaerobic activity, and where the activity and the intensity are irregular, a more sophisticated heart rate-derived methods, such as the modified heart rate-derived training impulse ($TRIMP_{MOD}$) have been developed (Foster et al., 2001; Stagno et al., 2007). Here, the intensity zones are weighed differently to account for the demands of anaerobic activity. A quite different method to quantify internal load is utilizing *Session rating of perceived exertion* (sRPE), a subjective rating of the intensity on a CR-10 scale, completed after the session, multiplied by the session duration, hence sRPE training load (sRPE-TL). The sRPE-TL method is an easy to use, low-cost method of measuring internal load that has been validated in football (Foster et al., 2001; Impellizzeri et al., 2004). It seems to have a stronger relationship with external load in football than for example TRIMP (McLaren et al., 2018). The reliability of sRPE-TL, on the other hand, is questionable. Reliability measurements from running (T. J. Scott et al., 2013) and cycling activity (Wallace, Slattery, Impellizzeri, & Coutts, 2014) have shown poor outcomes. Reliability testing in field settings is not straight forward to conduct due to difficulty to reproduce field sessions when players move freely. To date, reliability measurements from football field sessions are lacking.

Over the past decade, development and integration of player-tracking devices with GNSS and inertial measurement units (IMU) have made it easy to quantify external load with acceptable validity (Nicolella et al., 2018; Roell et al., 2018) and reliability (Luteberget & Spencer, 2017; Thornton et al., 2019). Player-tracking devices is typically worn in a vest and positioned between the shoulder blades (figure 2.2). GNSS tracks the position over time and therefore derives distance, speed and distance in speed zones metrics, whereas IMU has built-in accelerometer, gyroscope and magnetometers that tracks accelerations, decelerations, including their directions. The data can then be exported to computers where the whole team easily can be monitored.

Figure 2.2: Left: The S5 tracking device from Catapult Sports, with built-in GNSS, accelerometer, gyroscope and magnetometers. Right: The device is placed in pocket of a vest, with the device positioned between the scapulae.

Since the player tracking device only senses forces acting upon the device, external load measures do not consider the individual characteristics of the athlete, such as physiological-, morphological-, and mental factors or training

experience. As a result, a poorly trained athlete and well-trained athletes will have completely different internal response, i.e. HR, and sRPE-TL, to 1000 m of high-speed running. Furthermore, a tall, heavy central defender will likely not be able to perform as many accelerations and decelerations as a short, light central midfielder, holding the internal load constant. The potential differences between individuals, suggests that external load should be monitored individually and compared to themselves, since each player may have their own loading patterns.

Some other problems with the external load measurements arise due to the information extracted from these tracking device systems are divided into somewhat arbitrary external load *variables*. Examples of such variables are total distance covered, high-speed running distance (HSRD), PlayerLoad^{TM} (summation of accelerations in three dimensions), and number of accelerations and decelerations. Since any single external load variable covers only parts of the overall external load, they may vary on how they affect the internal load. An obvious example would be activity with jumping or fast change of directions, which is demanding for the muscles involved, but generating very little total distance. Furthermore, accelerations may no be equally taxing on the muscles involved depending on the directions of the accelerations (forward, sideways or backward). Lastly, these external load variables are linear measurements, but the stress impact on the tissues (muscle, tendon and bone) may not be the same for the 12 000 to 13 000 m part of the total distance covered as the firts 0 to 1000 m distance, nor will the impact of a sprint be immediatly after a high intensity period compared to after a low intensity period of the match. Hence, only considering one external load measure and treat it linearly may underestimate the true amount of load.

2.4 Relationship between internal and external load in football

Given the differences in internal and external measurements methods described in section 2.3, i.e. the objective but partial nature of external load, the individual response of internal load, and the ease of use of the tracking devices, it is important to fully understand the relationship between them. This is especially important as prescription of the same amount of external load can result in differences in internal load between players. In a meta-analysis comparing single external load variables to sRPE-TL in team sports, total distance covered ($r = 0.79$; 90 % confidence interval [CI], 0.74 to 0.83 %) and PlayerLoad^{TM} ($r = 0.63$; 90 % CI, 0.54 to 0.70 %) show the highest correlations, whereas HSRD ($r = 0.47$; 90 % CI, 0.32 to 0.59 %) and very high-speed running distance (VHSRD, $r = 0.25$; 90 % CI, 0.03 to 0.45 %) show lower correlations (McLaren et al., 2018). Attempts have previously been made to combine several external load variables to predict sRPE-TL in multiple regression analyses (Lovell et al., 2013), however, they explained no more variance than, for example, total distance or PlayerLoad^{TM} do alone (B. R. Scott et al., 2013). More research is needed to clarify these relationships. Interestingly, the magnitude of the correlation

coefficient seems to vary with training mode. McLaren et al. (2018) found that skills (enhance sport specific-skills) and neuromuscular (aims to increase force production/strength) training modes had possibly moderate to large reductions in the correlation coefficient compared with training sessions with a combination of two or more modes (mixed modes), while the difference between mixed and metabolic (aims to increase aerobic fitness) training modes was unclear. Due to differences in individual characteristics between players, several studies have chosen to analyze within-subject relationships between internal load and external load (Gaudino et al., 2015; Lovell et al., 2013). Nevertheless, little focus has been placed on how players differ in the relationship between external load and internal load. For example, individual players could vary in which external load variable was the most important descriptor of sRPE (Bartlett et al., 2017) implying that players have different internal load responses to the same external load variables. Given the large variation in player characteristics such as age, height, body mass, muscle strength, aerobic and aerobic capacity, that is not unlikely. The magnitude of the individual response to external load has, however, not been previously investigated.

2.5 Muscle structure and neuromuscular function

To manage the load of the individual players properly, it is important to know the relationship between external load and the individual response (internal load). However, it is equally important to know the relationship between the load and the time to *recovery* so that the right amount of load and rest can be prescribed. Football matches are known to result in long recovery times, for example, neuromuscular fatigue and physical performance impairments last typically up to 72–96 h post-match (Nédélec et al., 2012; Silva et al., 2018). These impairments may originate from both muscular and neural factors.

Human movements are produced when the muscles contracts and pulls on the skeleton, and the quality of the movement depends on the force of the contractions and the coordination of the muscles involved. A voluntary contraction of the muscle starts with a neural signal from the motor cortex or other supraspinal, corticospinal and propriospinal outputs, acting on the α and γ motoneurons in the spinal cord. If the motoneuron fires, the signal propagates through the motor axon which splits into branches and ends up in synapses with single muscle fibers. Acetylcholine is then released from the boutons in the neuromuscular junction and depolarize the muscle fiber and an action potential propagates along the sarcolemma and down through the T-tubules. When the action potential arrives at the excitation-contraction coupling, Ca^{2+} ions are released from the sarcoplasmic reticulum into the cytosol, where the Ca^{2+} ions binds to troponin C on the actin filament, freeing the binding places for myosin. The myosin then binds to the actin filament causing the muscle fiber, and muscle to contract. An overview of the steps in a voluntary contraction is given in figure 2.3 and the anatomy of the muscle fiber in figure 2.4.

The actin and myosin myofilaments together compose sarcomeres, which in

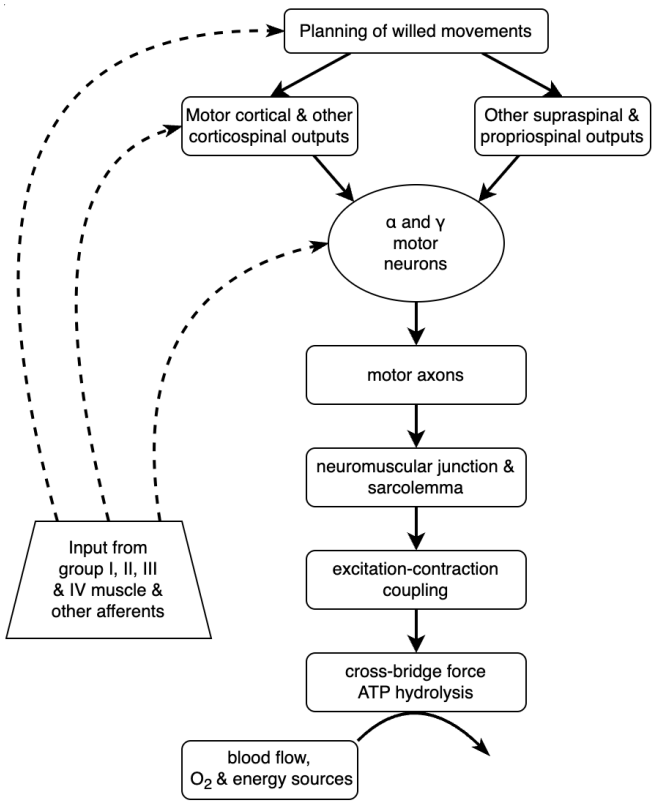


Figure 2.3: A diagrammatic representation of the steps involved in voluntary force production. Figure recreated from Gandevia, 2001

sequenced units forms myofibrils that can be seen with the recognizable striped appearance on a micrograph (figure 2.5). Myofibrils in parallel together compose a muscle fibre, which is surrounded by a cell membrane called sarcolemma. Muscle fibres are bundle together creating fascicles, which are bundle together again to form the entire muscle (figure 2.4). Muscle fibers are classified into slow-twitch Type-I fibers, and fast-twitch type II fiber types, where type II comes in two subtypes (IIA and IIX) based on expression of different isoforms of myosin heavy chain (Schiaffino & Reggiani, 1994). Human skeletal muscle contain all these three fiber types, but their proportions varies among different muscles and individuals. Type II fibers are characterized by high ATPase activity and high shortening velocity compared to type I fibers, whereas type I are more resistance to fatigue. The proportion of type I and II fibers in the muscles therefore determines the muscles properties in activity with high shortning velocity contractions such as sprinting and activity with long duration. Between the muscle fibers is a structurally stable composition of glucoproteins and collagen fibers called the extracellular matrix (ECM). It has a hierarchical organization with endomysium, perimysium and epimysium surrounding the muscle fibers,

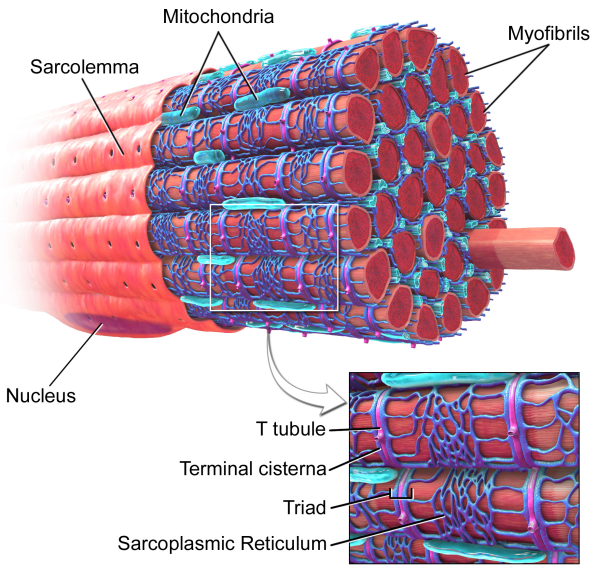


Figure 2.4: 3D-rendering of a skeletal muscle fiber, showing among other things sacolemma, myofibrils, t-tubuli, sarcoplasmic reticulum. The animation is downloaded from Blausen.com (2014)

fascicles and the muscle itself, respectively. The ECM, with it's connective tissue, contributes to the mechanical properties of the muscle tissue i.e. bears the majority of the passive load, but also plays an important role in muscle fiber force transmission from the myofilaments to the tenedon, maintenance, and repair of the fiber after damage (Gillies & Lieber, 2011).

2.6 Neuromuscular fatigue, muscle damage and recovery after football matches

Neuromuscular fatigue can be defined as an exercise induced reduction in the muscle's ability to exert force or power (Gandevia, 2001). It is neuromuscular because the reduction in force could be caused by both neurological factors including activation and propagation of the nerve impulse, and factors within the muscle itself. A sport specific test for measuring neuromuscular fatigue is the countermovement jump (CMJ) test, where the subject starts from an upright position and jumps as high as possible, preceding the jump by bending the knees (eccentric phase). Because jump height is dependent on the effect (maximal force production per time), any weakened part in the chain involved in voluntary contractions (figure 2.3) will result in a lower jump height. The similarity of the muscle groups involved and the explosive muscle contractions of the CMJ with running actions (especially accelerations efforts) in football play, makes the CMJ a specific and relevant test.

One cause of neuromuscular fatigue is suggested to be damage to structures

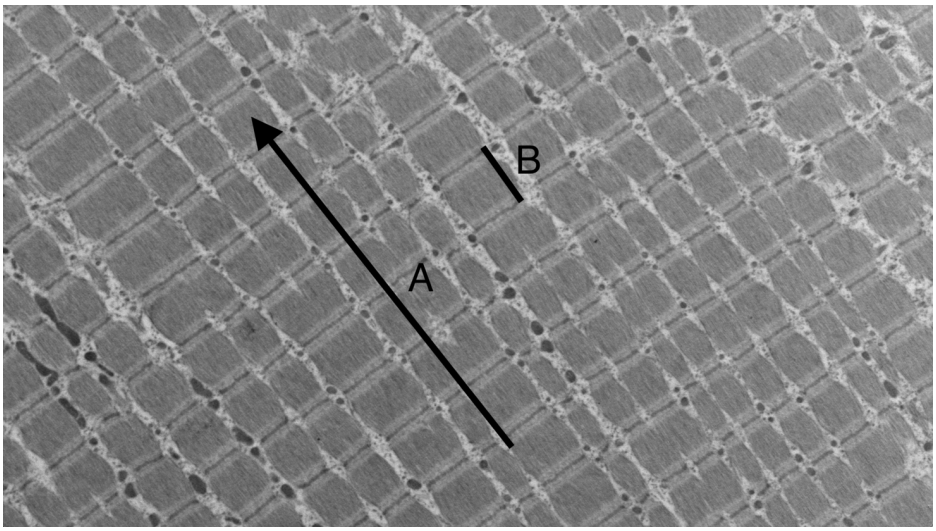


Figure 2.5: Electron micrograph showing the ultrastructure of a muscle fiber, with myofibrils in the longitudinal direction (A). The image constitutes about 20 myofibrils in transversal direction, which is about half the diameter of a single muscle fiber. The repeated areas between the dark lines (z-lines) on each myofibril are sarcomeres (B). The dark areas in the middle of a sarcomere is the A-bands where the actin and myosin cross-bridge activity takes place producing the contraction.

in the muscle cells. The evidence is based on a leakage of proteins, that normally is abundant and contained within the muscle cells, into the blood after muscle-damaging exercise. Two proteins that are commonly used as blood markers for muscle damage are creatine kinase (CK) and myoglobin. CK is an enzyme that catalyzes the conversion of creatine into phosphocreatine, which serves as an energy reservoir in the muscle. Myoglobin is a protein that serves as an oxygen reservoir in the muscle due to it's iron- and oxygen-binding characteristics. Blood levels of these two markers have shown great increase, concurrent with ultrastructural muscle damage observed on electron micrographs, and reduced muscular force, after muscle-damaging exercise protocols (Paulsen et al., 2007). Football match load is known to cause increases in muscle damage indicators (Andersson et al., 2008), as well as altering the biochemical milieu (Ascensão et al., 2008), and cause glycogen depletion (Bangsbo et al., 2006; Krustrup et al., 2022).

2.7 Effect of training- and match- load on neuromuscular fatigue and recovery

A few studies have investigated the relationship between external load variables and recovery from football matches via muscle damage indicators in blood and neuromuscular fatigue measurements (e.g da Silva et al., 2021; de Hoyo et al., 2016; Russell et al., 2016; Thorpe and Sunderland, 2012). While these studies have reported associations between post-match CK and high-intensity running

distance, sprint distance, and number of sprints, between post-match myoglobin and number of sprints, and between post-match CMJ and decelerations and accelerations, they are somewhat limited to correlation analyses with small sample sizes. Furthermore, from a practical point of view, there is a lack of studies investigating the specific effect of external load variables on recovery markers, both the magnitude of the effect and the recovery time back to baseline values. One exception is (Rowell et al., 2017) who found a dose-response relationship of PlayerLoad^{TM} on CMJ, but only one external load variable was investigated. Consequently, studies investigating several external load variables and their effect on important physical performance parameters such as sprint or intermittent running performance are needed.

Seventy-two hours post-match is a key time-point where the next match or a hard training session may take place. Most studies have examined these relationships for only 24–48 h post-match (da Silva et al., 2021; de Hoyo et al., 2016; Russell et al., 2016; Thorpe & Sunderland, 2012), despite evidence showing substantial changes in recovery markers at 72 h post-match (Ascensão et al., 2008; Ispirlidis et al., 2008). Additionally, due to individual differences in recovery time, some players might be recovered and some players not, hence being able to predict the recovery status for the individual player on day three post-match is practically important.

2.8 Ultrastructural muscle damage and Heat Shock Proteins

The post-match fatigue is thought to be caused by dehydration, glycogen depletion, mental fatigue, excitation-contraction coupling impairments, and muscle damage, where muscle damage is likely a major factor (Nédélec et al., 2012). The evidence for muscle damage is based on large increases in indirect markers for muscle damage such as blood concentrations of creatine kinase and myoglobin (Silva et al., 2013), increases in delayed onset of muscle soreness (Ispirlidis et al., 2008; Rampinini et al., 2011), swelling (Ispirlidis et al., 2008), and reduction in force-generating capacity (Krustrup et al., 2011) and power (Silva et al., 2013). However, studies from football matches documenting muscle damage at a cellular level are lacking and may increase the understanding of mechanisms behind the long recovery period after football matches.

Exercise-induced muscle damage is typically caused by unaccustomed muscle work, excessive force production, overstretching, and eccentric muscle actions (Paulsen et al., 2012). It is characterized by a decreased force-generating capacity, increase in muscle soreness, tissue swelling, and increases of muscle proteins in the blood such as creatine kinase and myoglobin (Clarkson & Hubal, 2002). At the cellular level, ultrastructural damage is characterized by cellular and sub-cellular disturbance, observed typically as z-line streaming (Fridén et al., 1981) and sarcomere disruptions (Raastad et al., 2010) on high magnification electron micrographs. Co-localized on the damaged myofibrils is heat shock proteins (HSP, Paulsen et al. 2009), a family of highly conserved proteins which functions as chaperones, helping to stabilize and refold damaged proteins.

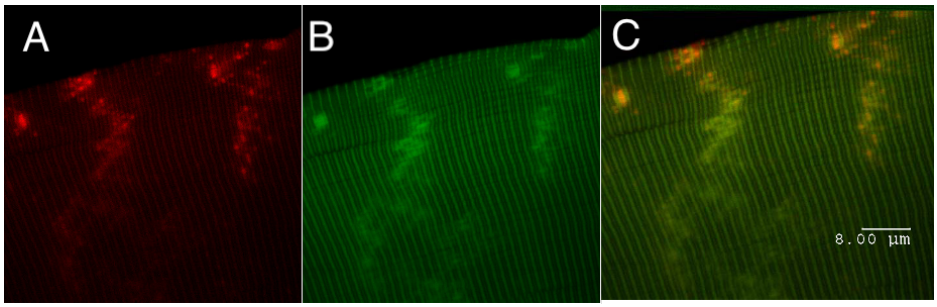


Figure 2.6: Image of a muscle fiber from m. biceps brachii 23 hours after eccentric, muscle damaging exercise. A) Staining of αB-crystallin, which seems to bind to z-disc-related structures, possibly to the intermediate filament protein desmin. B) Staining of actin filaments with phalloidin antibodies. C) Overlay of αB-crystallin and actin images. The images are taken with a confocal microscope by John Magne Kalhovde

Two of the commonly studied HSPs are αB-crystallin and HSP70. αB-crystallin is one of the small HSP (22 kDa) and seems to bind to z-disc-related structures, possibly to the intermediate filament protein desmin, after muscle-damaging exercise (Koh and Escobedo, 2004, and figure 2.6). The αB-crystallin response is rapid and can be observed within 0.5 h post-exercise (Paulsen et al., 2007). HSP70, which seems to be more involved in refolding and degradation of damaged proteins (Höhfeld et al., 2001), often has a more delayed and sustained response (Paulsen et al., 2007). Interestingly, exercise-induced muscle damage seems to lead to a translocation of the HSP from a soluble, unbound state in the cytosol, to binding to stressed structures of the cytoskeleton and sarcomeres (Cumming et al., 2014; Koh & Escobedo, 2004; Paulsen et al., 2009). After Western blotting, this is evident as a reduction in the amount of HSP in the cytosolic fraction and an concomitant increase in the cytoskeletal fraction. Furthermore, accumulation of the small HSP at disrupted sarcomeres has been observed via both electron and fluorescence microscopy, in the latter often as granular staining (Paulsen et al., 2009). Hence, the HSP response to exercise can be regarded as a proxy measure for ultrastructural muscle damage.

Exercise may also induce muscle damage to the passive extracellular structures. The ECM protein tenascin-c, which has de-adhesive function in remodeling of the ECM after muscle injury (Murphy-Ullrich, 2001), is rapidly up-regulated in the endomysium after increased loading on skeletal muscles (Hyldahl et al., 2015; Mackey et al., 2011) and has been suggested as an indicator of disruptions in the ECM (Crameri, Langberg, Magnusson, et al., 2004; Raastad et al., 2010). Tenascin-c could therefore be a marker for remodeling of ECM and should increase rapidly after damaging exercise.

In summary, there is growing literature on internal and external training load, the use of player tracking devices in load monitoring and recovery after football matches. What seems to be lacking is bridging the gap between external load variables and internal load variables, specifically how individuals respond differently to the same external load. Furthermore, identifying whether there is a dose response relationship between external load and recovery markers after

a football match is warranted. A last emerging question when we started this work was to what degree ultrastructural cellular damage occurs after football matches, and if such damages could explain why the recovery time after matches is so long. In the following section, the research aims and research questions are specified in more detail.

2.9 Purpose and aims

The purpose of this thesis was to explore the relationships between external load and internal load in elite football players. Furthermore, to investigate how external load from a football match affects the recovery of neuromuscular fatigue, muscle damage indicators, sprint performance and intermittent endurance performance, and investigate to what degree a football match results in cellular muscle damage. Specifically, the aims in the different papers included in this thesis were to:

Paper I:

1. Model the within-player and the between-player effects of different commonly used external load variables on sRPE-TL in elite football.
2. Model the magnitude of individual differences in sRPE-TL in response to external load.
3. Model the variability in sRPE-TL that is not explained by external load.

Paper II:

1. Investigate the recovery pattern of markers for muscle damage indicators (CK and myoglobin); neuromuscular function (CMJ); sprint performance (30 m sprint, SP30); and intermittent endurance performance (Yo-Yo Intermittent Recovery test level 1, YOYO).
2. Model the effect of external load variables such as playing duration, high-intensity events (HIE), HSRD, VHSRD, PlayerLoad^{TM}, and total distance covered on the recovery markers.
3. Investigate to what degree the recovery of individuals 72 h post-match can be predicted from external load variables.

Paper III:

1. Quantify the HSP response, as a proxy for muscle damage, in the cytosolic and cytoskeletal sub-cellular fractions and identify potential translocation from the cytosolic to the cytoskeletal fraction in the first 72 h after a football match.
2. Explore and compare the HSP response in type I vs type II muscle fibers after a football match.
3. Discuss the HSP response in relation to systemic markers of muscle damage such as creatine kinase, myoglobin, loss in power, muscle soreness, and match load.

Chapter 3

Methods

3.1 Study design and participants

To address the research aims in a best possible manner, two separate empirical studies were conducted. The relationships between internal and external load were investigated in Study I. Here an observational study design was applied where the participants' training load were measured over multiple training sessions within one competitive season. The results are summarized in Paper I. Possible relations between external load and the recovery of muscle function and performance after a single match, and to what degree the match results in cellular damage in muscle fibers, were investigated in Study II. An experimental, pretest-posttest design was applied, where the participants were tested before and at multiple time points after a football match. The results are summarized in Paper II. A subset of the participants in study II undertook muscle biopsies to further investigate cellular muscle damage, and these results are summarized in Paper III.

Table 3.1: Subject characteristics from Paper I–III. Values are mean ± SD.

Paper	Subjects	Level	Age (yrs)	Height (cm)	Body mass (kg)
I	18	Elite	25.7 ± 5.3	183.1 ± 5.9	80.2 ± 9.0
II	75	2.division	20.4 ± 4.7	177.7 ± 6.2	72.7 ± 7.3
III	12	2.division	19.4 ± 2.3	175.2 ± 5.7	71.4 ± 6.4

3.1.1 Study I (Paper I)

Participants

A total of 18 male players from one football team participating in the Norwegian Premier league, were included in the study. Subject characteristics are presented in table 3.1. The group of players included 7 defenders, 5 midfielders, and 6 attackers. Goal keepers were not included in the study due to their distinct activity profiles (White et al., 2018). All players provided written informed consent according to the Declaration of Helsinki. The study was approved by the Norwegian Centre for Research Data (Appedix A).

Study design

The study was designed to compare sRPE-TL and a variety of external load measures in professional football players, over a set of repeated training sessions. All sessions occurred during the in-season competition period, from March to

November (32 weeks). A total of 207 individual training observations from 21 training sessions were included, with a median of 10 ± 4 observations per player (range 7–18). While sRPE-TL was recorded after every training session of the season, recordings of external load was limited to one session per week due to availability of equipment and research staff. To be included in the study, a player had to have a minimum of 7 recorded sessions and played minimum one official premier league match. The inclusion criteria of minimum 7 completed sessions was set as a compromise between having enough data points per player and including enough players in the study.

All the training sessions were on-field sessions, with a duration of 75 ± 11 min (mean ± SD), excluding any individual preparations, warm-ups, or drills before and after the session. All sessions were performed on the same football pitch covered with third generation artificial turf. Most of the recorded sessions occurred on day three after a match (15), whereas three sessions occurred on day two, two on day four and one on day five after the previous match. The external load was recorded with the Catapult OptimEye S5 units during the session, whereas the sRPE-TL was recorded generally within one hour after the session via a Mobile-app. One player missed 5 sessions of HIE data, and another player missed one session with total distance data due to failure of two devices.

3.1.2 Study II (Paper II and III)

Participants

Eighty-one male football players from six Norwegian teams competing in the third highest national league in Norway participated in the study. The subject characteristics are summarized in table 3.1. The players reported an average of 7.6 ± 2.3 training sessions per week (matches excluded) for a typical in-season week. The number of players included in the different analyses is highlighted in figure 3.1 and table 3.3. Other than participating in the match, goal keepers were excluded from the analysis due to having a very different activity profile than outfield players (White et al., 2018).

A subset of twelve participants (age 19.4 ± 2.3 y, height 175 ± 6 cm and body mass 71 ± 6 kg) from five of the teams, 1–3 from each team, volunteered to donate muscle biopsies. The group of players consisted of 2 attackers, 2 central defenders, 4 central midfielders, 3 full backs, and 1 wide midfielder. The self-reported number of training sessions for a typical in-season training week was 8 ± 2. In the last week before their experimental match, 50 % of the players reported to have "somewhat less" and 25 % reported "less" training sessions than a typical in-season week. All players were informed about potential risks and gave written informed consent before commencing the study. The study was approved by the Regional Ethics Committee of South-East Norway (Appendices B and C).

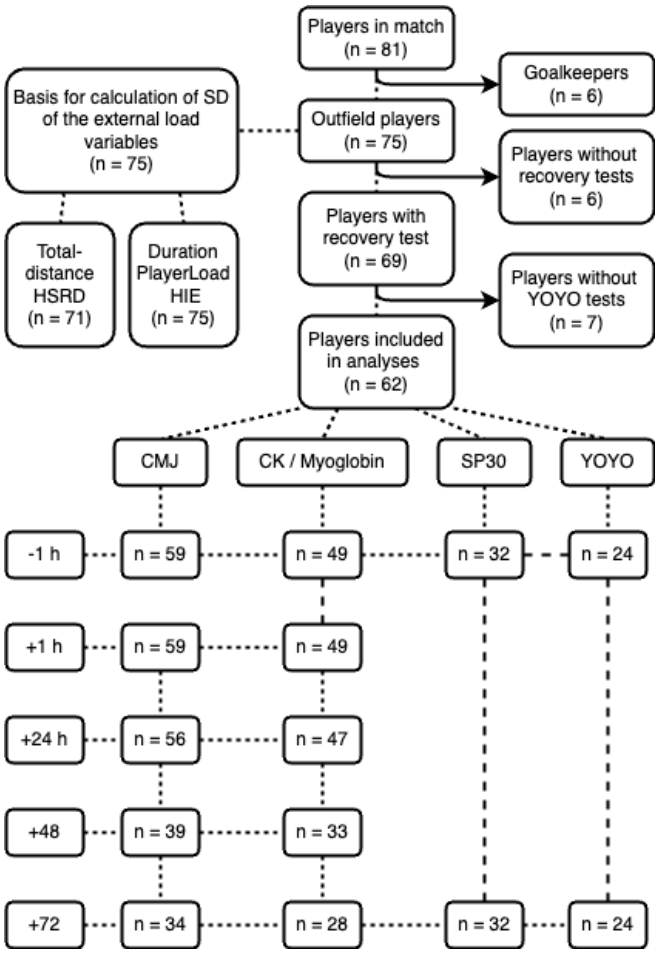


Figure 3.1: Flow chart showing number of participants included in the analyses of the different recovery markers, on each time-point, and in the calculation of SD used for the rescaling of the external load variables.

Study design

The study was designed to investigate how a group of football players' external load in a single football match affected physical performance and a set of muscle damage measurements in a 72 h post-match period. The study took place 14–23 days after their last match of the season. The six club teams were set up by the investigators to form three experimental matches (one match per team), where the opponents were considered as rivals. All matches were played in the same indoor football stadium (105×65 m), covered with a 3rd generation artificial turf, a type of turf that was common for all players. The air temperature was $14.5 \pm 1.1\,^{\circ}$C and the relative humidity were 70 to 83 %. The baseline and post-match muscle biopsies, blood samples, CMJ, and perception of muscle soreness were obtained -1, 1, 24, 48 and 72 h relative to the matches, except for

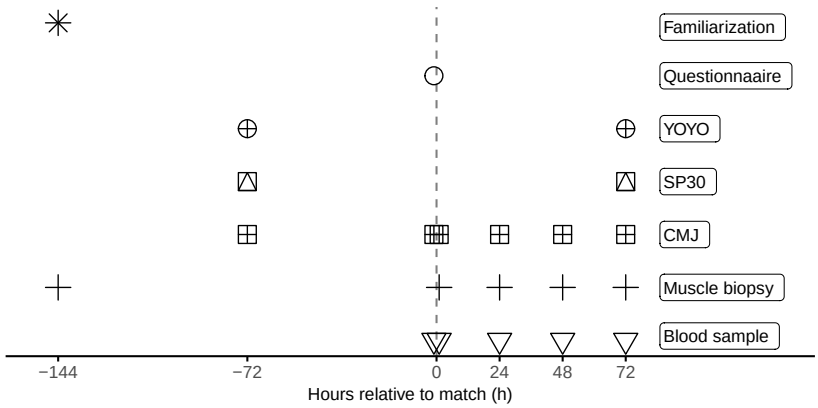


Figure 3.2: Timeline of the measurements in study II. The time-points indicates hours before the start of the match, and hours after the end of the match.

the baseline muscle biopsies which were obtained 3 to 6 days before the match (Figure 3.2). A session with familiarization to the CMJ, SP30, and YOYO procedures were conducted 6 days before the matches. When conducted on the same day, the test order was: biopsies, blood samples, CMJ, SP30, and YOYO. The players were instructed to refrain from other intense physical exercises within the study period and to follow their normal preparation before the match regarding nutrition and sleeping strategies.

The matches were preceded by a standardized 40 min warm-up consisting of 5 min of jogging, the CMJ test procedure, team-organized running drills, and a play exercise. Standard 90 min matches were officiated according to FIFA rules, and teams and players were instructed to give their best to win. Immediately after the match, the players consumed a 330 mL recovery drink (Yt Restitusjonsdrikk, TINE, Norway) containing 30 g carbohydrate, 20 g protein, and 3.5 g fat. They then completed a questionnaire. In accordance with the study objectives and typical substitution practices in official matches, 2 to 3 pre-planned substitutions at 45 and 60 min were implemented per team to spread the match load from low to high values, see figure 4.3.

3.2 Data collection

The following description of the test procedures applies to both studies, unless otherwise is specified.

3.2.1 Tracking of External Load (Study I and II)

Two different measurement systems for external load where utilized across the two studies based on the external conditions. In Study I, where the training sessions occurred outdoor, a Global Navigation Satellite System (GNSS) was utilized. The matches in Study II were played indoors where GNSS doesn't

Table 3.2: Descriptions of the External Load variables and measurement devices used in Study I and II

External Load Variable	Unit	Cutoff	Intensity-threshold[a]	Devices in Study I	Devices in Study II
Duration	minute			*OptimEye S5*	*OptimEye S5*
HIE > 1.5[b]	$\mathrm{m\,s^{-1}}$	$\geq 1.5\,\mathrm{m\,s^{-1}}$	low	*OptimEye S5*	
HIE > 2.5[b]	$\mathrm{m\,s^{-1}}$	$\geq 2.5\,\mathrm{m\,s^{-1}}$	high	*OptimEye S5*	*OptimEye S5*
HIE > 3.5[b]	$\mathrm{m\,s^{-1}}$	$\geq 3.5\,\mathrm{m\,s^{-1}}$	high	*OptimEye S5*	
HSRD[c]	m	$\geq 4.0\,\mathrm{m\,s^{-1}}$	high	*OptimEye S5*	
PlayerLoad$^{\mathrm{TM}}$	AU[e]		low	*OptimEye S5*	*OptimEye S5*
PlayerLoad2D$^{\mathrm{TM}}$	AU[e]		low	*OptimEye S5*	
Total Distance	m	$\geq 0\,\mathrm{m\,s^{-1}}$	low	*OptimEye S5*	*ClearSky T5*
VHSRD[d]	m	$\geq 5.5\,\mathrm{m\,s^{-1}}$	high	*OptimEye S5*	*ClearSky T5*

[a] Only work over this intenisty-threshold is measured [b] High Intensity Events
[c] High-Speed Running Distance [d] Very High-Speed Running Distance [e] Arbitrary Units

work, thus a Local Positioning System (LPS) was used to acquire external load variables based on position. However, the LPS system did not have the external load variables based on accelerometers, so both systems were used together with GNSS sensors turned off.

Global Navigation Satellite System and Inertial Measurements Units

Each player (both studies) was equipped with a tracking device (OptimEye S5, Firmware 7.18; Catapult Sports, Melbourne, Australia), located between the scapulae in a manufacturer-provided vest. The device was specified with a non-differential, 10 Hz GNSS and a 3-dimensional accelerometer, magnetometer, and gyroscope, all operating at 100 Hz. There was some interchange of devices between players in Study I across time, resulting in that players used the same device for approximately 50 % of the sessions. All the devices were calibrated according to the manufacturer's instructions prior to the commencement of both studies. In Study I, the devices were turned on outdoors, 15 min minutes before data collection commenced, and in Study II the devices were turned on indoors with the GNSS sensors turned off. We extracted the raw data from the tracking devices after each session using the Catapult Sprint software (version 5.1.7; Catapult Sports).

For Study I, eight different variables were extracted from the software to provide different representations of the actual external training load (table 3.2). PlayerLoad$^{\mathrm{TM}}$ is a vector magnitude expressed in arbitrary units as the square root of the sum of the squared instantaneous rate of change in acceleration in 3 dimensions, described more comprehensively by Boyd et al. (2011). PlayerLoad2D$^{\mathrm{TM}}$ excludes the vertical dimension. High-intensity events (HIE) are the sum of acceleration, deceleration, and change of direction events exceeding

a threshold of either $1.5\,\mathrm{m\,s^{-1}}$ (HIE > 1.5), $2.5\,\mathrm{m\,s^{-1}}$ (HIE > 2.5), or $3.5\,\mathrm{m\,s^{-1}}$ (HIE > 3.5), based on procedures by Luteberget and Spencer (2017). During indoor field assessment, PlayerLoad$^{\mathrm{TM}}$, PlayerLoad2D$^{\mathrm{TM}}$, HIE > 1.5, HIE $>$

2.5, and HIE > 3.5 have shown a between-device coefficient of variation (CV) of 0.9%, 1.0%, 1.8%, 3.1%, and 5.5%, respectively (Luteberget et al., 2018). Three variables of total distance covered were categorized into total distance ($> 0\,\mathrm{m\,s^{-1}}$), High speed running distance (HSRD, $> 4\,\mathrm{m\,s^{-1}}$), and Very High Speed Running Distance (VHSRD, $> 5.5\,\mathrm{m\,s^{-1}}$). The between-device reliability of total distance variables with different thresholds have been estimated with CVs of 1.5% ($> 0\,\mathrm{m\,s^{-1}}$), 0.6% (3–$5\,\mathrm{m\,s^{-1}}$), and 1.0% ($> 5\,\mathrm{m\,s^{-1}}$) (Thornton et al., 2019).

Of these eight, PlayerLoad$^{\mathrm{TM}}$, PlayerLoad2D$^{\mathrm{TM}}$, total distance, and HIE > 1.5 were regarded as variables with *low intensity-thresholds*, meaning that motions with low and high intensity were acquired. The remaining four, HSRD, VHSRD, HIE > 2.5, and HIE > 3.5 were regarded as variables with *high intensity-thresholds*, meaning that only high intensity motions were acquired.

Local Positioning System

Because of the indoor environment in Study II, the players wore one LPS device (ClearSky T5, Catapult Sports, Australia) in addition to the IMU device (OptimEye S5, with GNSS turned off). The devices were taped together, with the IMU closest to the body and located between the scapulae in a manufacturer provided vest (Catapult Sports, Australia). The LPS was set up with 18 anchor nodes fixed around the pitch, and spatial calibration was carried out according to manufacturer's recommendations. Three players missed LPS data due to signal problems and one due to limited available LPS devices. Raw data was extracted from the LPS using the Openfield Software (version 1.12, Catapult Sports, Australia).

In Study II, five different external load variables were chosen to provide different representations of the actual match load (table 3.2). Total distance and VHSRD were taken from the LPS, whereas Playing Duration (on field time), HIE > 2.5, and PlayerLoad was taken from the IMU's. Total distance ($> 0\,\mathrm{m\,s^{-1}}$) and VHSRD ($> 5.5\,\mathrm{m\,s^{-1}}$) from the ClearSky T5 is equivalent to the OptimEye S5, although measured with LPS technology instead of GNSS technology. The concurrent validity between the two system was not tested. A validity study using the same LPS system as the current study has shown a 2–4 % error in linear and nonlinear distance when conducted in an indoor environment (Sathyan et al., 2011).

3.2.2 Internal load: Session Rating of Perceived Exertion (Study I)

Each player reported their sRPE via a mobile app (PMSYS; University of Oslo, Oslo, Norway) on their private phone 45 min (median) after the session. The mobile app presented a modified Borg CR-10 scale (Foster et al., 2001) with integers scale combined with verbal anchors (figure 3.3). sRPE-TL was calculated by multiplying the sRPE by the session duration in minutes. Although the players reported session duration in the app, we defined session duration from the start and stop in the tracking system recordings to be more accurate

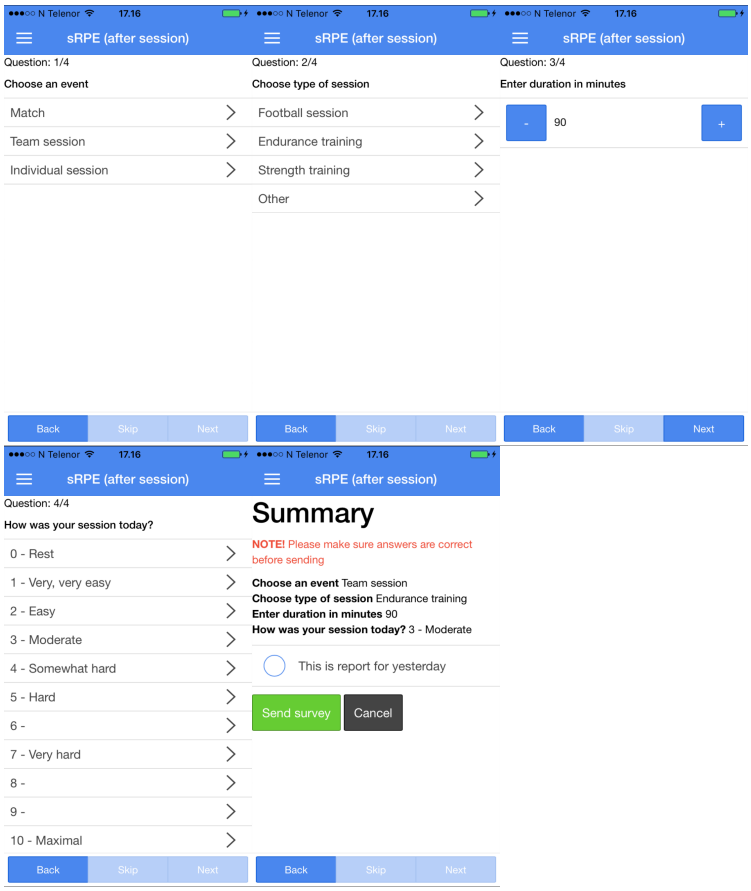


Figure 3.3: Screenshots from the app used for collection of sRPE after the training sessions in study I, including the sRPE scale with verbal anchors

and consistent. That means that any individuals activity before or after were excluded from the duration measurements. We instructed the players to consider each training session as multiple small periods with a hypothetical rating of perceived exertion (RPE) of each period. The sRPE should represent an average of all the hypothetical RPEs throughout each session. The players did not know the outcome from the external load measurements before they reported their sRPE. The player's were familiarized with sRPE, and had been using regularly for 27 ± 18 days (range 10–89 days) before their first included session.

3.2.3 Neuromuscular function and performance tests (Study II)

Three different football specific performance tests were chosen to measure a variety of neuromuscular function pre- and post-match in study II; CMJ to cover explosive, vertical, maximally voluntary force, SP30 to cover horizontal acceleration and speed and YOYO to cover football specific endurance.

Countermovement jump (CMJ)

The CMJ procedure was performed on a portable force platform (FP4, HUR labs, Tampere, Finland). The participant started and ended in an upright, standing position in the middle of the platform, with his hands placed on the hips throughout the whole procedure. The jump was initiated with a descending (eccentric) phase, down to about 90° angle in the knee joint, before the ascending (concentric) jump. The participants went trough a familiarization session with instructions and practice to optimize their jumping technique before the first test, 72 h before the match. Jump height in cm was recorded and calculated based on takeoff velocity by the software provided by manufacturer (Force Platform Software Suite, Version 2.6.51, Kokkola, Finland).

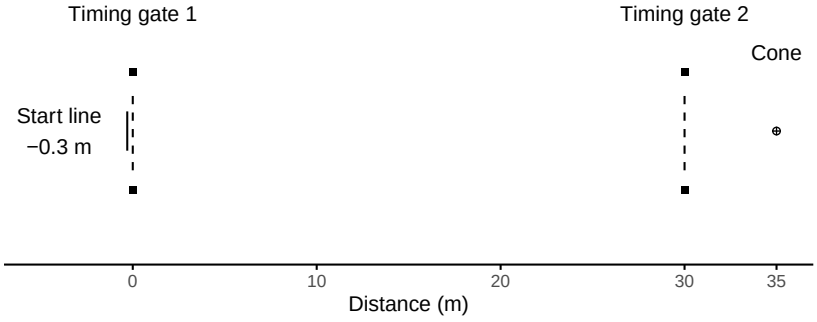
Data from our lab show a CV of 4.7 %. The warm-up procedure consisted of a 5 min jog followed by three jumps with 80, 90 and 100 % effort. Each player performed three to five jumps, interspersed with 15 s of rest, where the highest jump was used for analyses. The best of the −72 and −1 h CMJ was used as the baseline value.

30-m sprint (SP30)

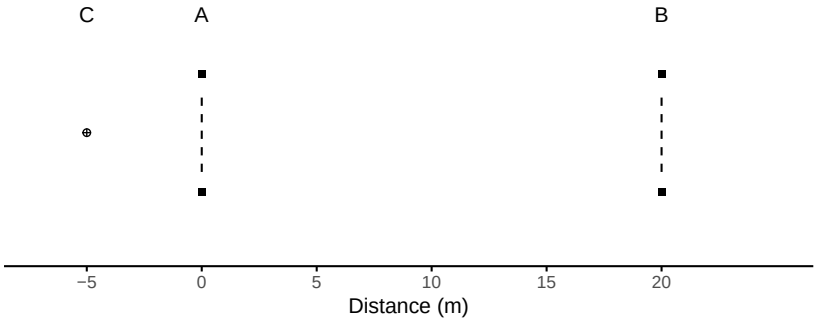
SP30 was measured with error correction processing timing gates (SmartSpeed Pro, Fusion Sport, Brisbane, Australia) placed at 0 and 30 m, and with a starting position 0.3 m before the first gate (figure 3.4a). Participants were instructed to start in a static, forward leaning position, and then sprint as fast as possible past a cone placed at 35 m. Three trials per participant, with minimum 2 min of rest between, were recorded and the best exported for analysis. Reliability testing from our lab shows a CV of 1.7 % for the sprint procedure. Baseline values were taken from the −72 h SP30 test.

Yo-Yo Intermittent Recovery Test Level 1 (YOYO)

As a measure of football specific endurance performance, we utilized the Yo-Yo Intermittent Recovery test level 1 (YOYO). The test is a 2×20 m back and forth shuttle-run, with 10 s active rest (2×5 m) between each bout. The speed is controlled by audio beeps and is progressively increased until the subject is exhausted, i.e. when the participants fail to reach the finish line two times (figure 3.4b). A complete instruction of the test is stated in Krustrup et al. (2003). A standardized warm-up consisting of the 11 first stages of the test followed by a 2 min rest were undertaken, before the test. The total distance in meters was used in the analysis. Baseline values were taken from the −72 h YOYO test, and the best of the pre- and post-results (YOYOmax) was used as a measure of the players' overall aerobic fitness. The test-retest CV is shown to be 4.9 % (Krustrup et al., 2003).



(a)



(b)

Figure 3.4: (a) Schematic of the 30 m sprint test. The participants starts 0.3 m before the first timing gate, and run past the cone at 35 m. (b) The YOYO Intermittent Recovery Test Level 1 consists of a shuttle-run between line A and line B, with 10 s rest while walking around a cone (C).

3.2.4 Muscle damage: indirect blood markers (Study II)

Venous blood samples were drawn from the inside of the elbow. The samples were then centrifuged at 4 °C for 10 min at 1300 g and stored in −80 °C until analyzed for CK and myoglobin at the Oslo University Hospital, Rikshospitalet (Oslo, Norway; Cobas 8,000, Roche Diagnostics, USA). The laboratory's stated CV is 5 % for CK and 6 % for myoglobin. Baseline values were taken from the −1 h blood sample.

Table 3.3: Sample size for each outcome measure and time point (baseline n = 12). Several biopsy analyses were excluded due to poor muscle tissue quality while some players omitted the biopsy procedure at 48 h

Outcome variable	Structure	1 h	24 h	48 h	72 h
αB-crystallin	Cytosolic	12	12	7	11
αB-crystallin	Cytoskeletal	11	10		9
αB-crystallin	Type I & II	12	12	7	11
HSP70	Cytosolic	12	12	7	11
HSP70	Cytoskeletal	12	12		11
HSP70	Type I & II	12	12		11
Granular αB-crystallin		10	11		9
Tenacin-C		12	12		11
Creatine Kinase & myoglobin		11	11	10	11
CMJ		11	11	9	11
Muscle soreness			11	8	

3.2.5 Muscle damage: heat shock protein analyses on muscle biopsies (Study II)

Acquisition of muscle samples

Muscle biopsies were obtained from the mid-portion of m. vastus lateralis from the participants' dominant leg (baseline, 1 and 72 h time points), and from their non-dominant leg (24 and 48 h time points). The insertions of the repeated biopsies were placed 3 cm proximally from the previous biopsy to minimize any impact of the procedure itself on the muscle samples. The procedure was performed under local anesthesia (Xylocain adrenalin, $10\,\mathrm{mg\,mL^{-1}} + 5\,\mu\mathrm{g\,mL^{-1}}$; AstraZeneca, London, UK), and approximately 200 mg (2–3 × 50–150 mg) of muscle tissue was obtained with a modified Bergström needle using the suction technique. The portion of muscle tissue used for homogenization was quickly rinsed in physiological saline before visible fat, connective tissue, and blood were removed. The sample weight was recorded before the tissue was frozen in dry-ice-cooled isopentane. A separate muscle tissue sample, for immunohistochemistry, was mounted in a OCT embedding matrix (KMA-0110-00A, CellPath, Newtown Powys, UK) and quickly frozen in isopentane, pre-cooled on liquid nitrogen to the freezing point. All muscle samples were stored at −80 °C until further analyses.

Quantification of HSP by Western blot

Approximately 50 mg of muscle tissue was homogenized and fractionated into cytosolic, nuclear, membrane, and cytoskeletal fractions using a commercial fractionation kit (ProteoExtract Subcellular Proteome Extraction Kit, 539790, Calbiochem, EMD Millipore Corporation, Billerica MA, USA). Protein concentration was measured using a commercial kit (Bio-Rad DC protein assay, 0113, 0114, 0115; Bio-Rad Laboratories, Inc., Hercules, CA, USA) and measured by colorimetric intensity using a filter photometer (Expert 96, ASYS Hitech GmbH, Ec, Austria). Protein concentration was calculated using Kim32 software

(Kim Version 5.45.0.1, Dan Kittrich, Prague, Czech Republic). The cytosolic and cytoskeletal fractions were analyzed by Western blotting. Between 6 and 24 µg of denatured proteins, depending on the sub-cellular fraction, were separated by electrophoresis through 4 to 20 % gradient gels (Mini-PROTEAN® Stain-FreeTM Gels, 456–8094, Bio-Rad Laboratories, Inc.) under denaturing conditions at 200 V for 30 min in running buffer (10x TGS Buffer, 1610732; Bio-Rad Laboratories GmbH, München). Proteins were then transferred to PVDF membranes (Immun-Blot PVDF, 162–0177; Bio-Rad Laboratories, Inc.), which were immersed in a blocking solution consisting of 5 % fat-free skimmed milk in tris-buffered saline (TBS) with 0.1 % Tween-20 (TBS-T; TBS, 1706435, Bio-Rad Laboratories, Inc.; Tween-20, 437082Q, VWR International, Radnor, PS, USA; skim milk powder 1.15363, Merck KGaA, Darmstadt, Germany) for 2 h at room temperature. Blocked membranes were incubated with primary antibodies (table 3.4) against αB-crystallin or HSP70 (Enzo Life Sciences, Inc., Farmingdale, NY, USA) over night at 4 °C with gentle agitation. Incubation with horseradish peroxidase-coupled secondary antibodies (Goat anti-Mouse IgG Thermo Fisher Scientific, Inc., Hanover Park, IL, USA) was done for 1 h at room temperature with gentle agitation. All antibodies were diluted in TBS-T with 1 % fat-free skimmed milk. Between stages, the membranes were washed with 0.1 % TBS-T. An HRP-detection system was used to visualize protein bands (Super Signal West Dura Extended Duration Substrate, 34076, Pierce Biotechnology, Thermo Scientific, Rockford, IL, USA). Quantification was done using the ChemiDoc™ MP (Bio-Rad Laboratories, Inc.) detection system. Protein band intensities were calculated using Image Lab software (version 5.1, Bio-Rad Laboratories, Inc.). All protein band intensity measurements were normalized to the amount of protein measured in the membrane after blotting (Gürtler et al., 2013).

Quantification of HSP by Immunohistochemistry

Eight µm thick cross-sections were cut with a microtome at −20 °C (CM1860 UV, Leica Microsystems GmbH, Nussloch, Germany) and mounted on microscope slides (Superfrost Plus, Thermo Fisher Scientific, Inc., Waltham, MA, USA). All cross-sections from the same participants were arranged on the same slides. The sections were air-dried and stored at −80 °C until further analysis. The αB-crystallin, HSP70, muscle fiber type, and tenascin-C analyses were conducted on separate cross sections. All cross sections were blocked in room temperature with 2 % bovine serum albumin (BSA; A4503, Sigma Life Science, St Louis, MO, USA) in phosphate-buffered saline (PBS) with 0.05 % Tween-20 (PBS-T; PBS, 524650, Calbiochem, EMD Biosciences). The sections were incubated with the analysis-specific primary mouse monoclonal antibody, and an additional primary rabbit polyclonal dystrophin antibody (Abcam, Cambridge, UK) to stain the sarcolemma. Lastly, appropriate secondary antibodies (anti-rabbit Alexa Fluor 594 and anti-mouse Alexa Fluor 488, Life Technologies, Invitrogen, Rockford, IL, USA) were applied to the sections before incubation for 60 min in room temperature. All antibodies were diluted in the blocking buffer with a specific dilution ratio listed in (table 3.4). Between stages, the sections were washed

Table 3.4: Primary and secondary antibodies for Western blotting and immunohistochemistry, along with catalog number, concentrations, and applied buffer dilutions

Antibody	Cat#	Concentration	Dilution
Western blot:			
αB-crystallin[a]	ADI-SPA-222 F	$1\,\mathrm{mg\,mL^{-1}}$	1:4000
HSP70[a]	ADI-SPA-810 F	$1\,\mathrm{mg\,mL^{-1}}$	1:4000
Goat anti-Mouse IgG[b]	31430	$1\,\mathrm{mg\,mL^{-1}}$	1:30 000
Immunohistochemistry			
αB-crystallin[a]	ADI-SPA-222 F	$1\,\mathrm{mg\,mL^{-1}}$	1:200
HSP70[a]	ADI-SPA-810 F	$1\,\mathrm{mg\,mL^{-1}}$	1:200
Tenacin-C[c]	MA5-16086	$1\,\mathrm{mg\,mL^{-1}}$	1:100
SC-71[d]			1:500
Dystrophin[e]	Ab15277		1:500
Secondary antibodies			
Alexa Fluor 594[f]	A11005	$2\,\mathrm{mg\,mL^{-1}}$	1:200
Alexa Fluor 488[f]	A11001	$2\,\mathrm{mg\,mL^{-1}}$	1:200

[a] Enzo Life Sciences, Inc., Farmingdale, NY, USA.
[b] Thermo Fisher Scientific, Inc., Hanover Park, IL, USA.
[c] Thermo Scientific, Rockford, IL, USA.
[d] Schiaffino, S., obtained by DSHB, Iowa, IA, USA.
[e] Abcam, Cambridge, UK.
[f] Life Technologies, Invitrogen, Rockford, IL, USA.

3×5 min (3×10 min for tenascin-C) in PBS-T. The sections were embedded in ProLong Gold Antifade Reagent with DAPI (P36935; Invitrogen Molecular Probes, Eugene, OR, USA) and left to cure overnight at room temperature. For optimal staining on the sections, the primary antibodies (table 3.4) required different BSA-blocking and incubation steps.

Sections with HSP70 antibodies were blocked for 30 min and incubated for 2 h room temperature. αB-crystallin sections were blocked for 60 min (10 % goat serum was added to the blocking buffer to reduce background noise) and incubated overnight at 4 °C. Sections with myosin-heavy chain type II antibodies (SC-71, developed by Schiaffino, S., obtained by DSHB, Iowa, IA, USA) were blocked for 30 min and incubated for 60 min at room temperature. Finally, the tenascin-C sections were fixed in 2 % paraformaldehyde for 5 min followed by 10 min permeabilization in 0.2 % triton X-100 in PBS, before blocked in 2 % BSA with 5 % goat serum in PBS-T, for 60 min. The sections were incubated in antibodies against tenascin-C (Thermo Scientific, Rockford, IL, USA) overnight at 4 °C. Images of the muscle sections were acquired using a high-resolution camera (DP72, Olympus Corp., Tokyo, Japan) mounted on a microscope (BX61, Olympus Corp., Japan) with a fluorescence light source (X-Cite 120PCQ; EXFO Photonic Solutions Inc., Mississauga, ON, Canada). Camera and software settings were fixed to be able to compare staining intensities between muscle sections within the same participants. Quantification of staining intensity was conducted using the Fiji distribution of ImageJ (Schindelin et al., 2012), where the researcher was blinded for subject and time point. For the HSP staining intensity analyses, a single image was acquired with a total of 213 ± 52 (range 73–322) fibers analyzed per cross-section. The fibers were related to their respective muscle fiber type (from a separate, sequential section) and average

staining intensity per fiber type was calculated. Of these fibers, $70 \pm 9\,\%$ were type II. An increase in HSP staining intensity indicates bound proteins to cytoskeletal structures. Analysis of granular staining was conducted manually by eye, by determining the proportion of fibers with αB-crystallin granule stains in proportion to all the fibers. Here, multiple images with a total average of 880 ± 397 fibers (range 168–2176) were analyzed per section. Ruptured fibers and the outermost layer of the muscle section were excluded. For the tenascin-C analysis, one image was acquired per section, covering most of the muscle sample. An optimal signal-to-noise ratio for positive staining was set in the Fiji software and used for all images, and the percentage of the total cross-section with positive tenascin-C staining was calculated.

3.2.6 Questionnaire (Study II)

Fifteen minutes after the end of the matches in Study II, each participant completed a short questionnaire. The aim was to subjectively compare the experimental match with a typical in-season match. The questions aimed to function as control questions to address that we were not able to study an in-season competitive match. The specific questions where:

1. How many training sessions do you have during a typical in-season week (including team and individual sessions)?
2. How much was your training load the last week before the match, compared to a typical training week?
3. How fatigued were you compared to a typical in-season match?
4. How much did you run compared to a typical in-season match?
5. How was your overall performance compared to a typical in-season match?

Except for question 1, the questions were answered on a 1–5 Likert scale where the levels were as follow: Less, somewhat less, same, somewhat more, more.

3.3 Statistics Analyses

Due to the repeated measurement design of the studies, the data in all three papers were analyzed using linear mixed-effects models (The MIXED procedure in SAS software, version 9.4; SAS Institute, Cary, NC). Mixed-effects models incorporates both fixed and random effects. Random effects can be specified to adjust for correlations, i.e. repeated measurements on the same subject are correlated, and that the repeated measurements may have unequal variances (Paper II and Paper III). Random effects can also be specified to estimate the variability within and between subjects, variability in the response to x, and variability between training sessions (Paper I).

The response variables in all models were log-transformed to the natural logarithm due to the residuals having a log-normal distribution. Because log-transformation of the response variable makes the relationships with it's predictors multiplicative, all effects were back-transformed to percent or factor effects. The results are presented as point estimates with CI, and standardized effect sizes to assess the magnitude of the effects. Additional statistical inferences are provided by non-clinical magnitude-based inferences (MBI; Hopkins et al., 2009) in Paper I and Paper II and p-values in Paper III.

3.3.1 Statistical model in Paper I

In Paper I, sRPE-TL was predicted by the external load variables, and separate analyses was conducted for each predictor variable. The HSRD and VHSRD predictors were log-transformed to address non-linearity, i.e., many observations with small values, and few with large values. Two fixed-effect parameters were specified to separate within-player and between-player effects of the external load variable on sRPE-TL. To obtain the within-player effect, the external load variable was centered to the mean of each player. To obtain the between-player effect, the individual player's mean external load of all sessions was repeated for each observation of sRPE-TL. The model was specified with random intercept for Player ID and random slope for Player ID $\times$ predictor (with an "unstructured" covariance structure), as well as random intercept for Session ID. We allowed for negative variances to estimate realistic confidence limits for the variances and the SD derived. The random effects are presented as SD (in percentage) and represent pure between-player variability (Player ID), individual response of the predictor (Player ID $\times$ predictor), between-session variability (Session ID), and within-player variability in a typical session (residuals). The predictors were centered and rescaled to a SD of 0.5 to properly evaluate the magnitude of the effect of continuous variables (Gelman, 2008; Hopkins et al., 2009). A 2-SD gauge of the effects can be justified as the difference between a typical high and a typical low load training session (within-player fixed effect), and the difference between players with typical high and a typical low average external load (between-player fixed effect). The magnitudes of the effects are presented as standardized effect sizes (the effects divided by the square root of the sum of the Player ID and residual variances), where <0.2, 0.2–0.6, 0.6–1.2, 1.2–2.0, and >2.0 are regarded as trivial, small, moderate, large, and very large effects, respectively. For interpreting random effects, which are SDs, these thresholds are halved (Hopkins et al., 2009). Nonclinical, MBI were used, where an effect was deemed unclear if the 90 % CI included small positive and negative effects; the effect was otherwise deemed clear. Qualitative assessment of chances of clear outcomes was as follows: >25 to 75 %, possibly; >75 to 95 %, likely; >95 to 99 %, very likely; >99 % most likely (Hopkins et al., 2009)

3.3.2 Statistical model in Paper II

In Paper II, the recovery markers were modelled as change-scores, with Time, Time × external load variable, Time × baseline, and Time × YOYOmax specified as fixed effects. Time was treated as nominal variable. When YOYO was the dependent variable, YOYOmax was omitted from the model because it contained partly the same numbers as YOYO baseline. To deal with interdependency and unequal variances in the models with repeated measurements (CK, myoglobin, and CMJ), the R matrix were specified with Time, Player ID as blocks and an "unstructured" covariance structure, using the REPEATED statement in the MIXED procedure in SAS. SP30 and YOYO had no repeated measurements and were analyzed without a REPEATED statement. Separate analyses were done for each external load variable for every recovery marker. The main effect of interest, Time × match load, was adjusted for baseline to address the regression to the mean effect, and YOYOmax to address the possibility of fitness being a confounder affecting both match load (Bradley et al., 2013; Krustrup et al., 2003; Redkva et al., 2018) and recovery (Johnston, Gabbett, Jenkins, & Hulin, 2015). Furthermore, to properly evaluate the magnitude of the effect of continuous variables, they were rescaled by dividing by two standard deviations (SDs). Two SDs also correspond approximately to the mean separation of lower and upper tertiles (Hopkins et al., 2009), and can be justified as a separation of typically high and low match loads. The magnitude of the effects is presented as standardized effect sizes (ES: the effects divided by the SD of the baseline value), where <0.2, 0.2–0.6, 0.6–1.2, 1.2–2.0, and >2.0 are regarded as trivial, small, moderate, large, and very large effects respectively. Nonclinical, MBI were used, where an effect was deemed unclear if the 90 % confidence interval included small positive and negative effects; the effect was otherwise deemed clear. Qualitative assessment of chances of clear outcomes were as follows: >25 to 75 %, possibly; >75 to 95 %, likely; >95 to 99 %, very likely; >99 % most likely (Hopkins et al., 2009)

3.3.3 Statistical model in Paper III

In Paper III, the outcome variables were analyzed as change scores with Time and Time × Baseline specified as fixed effects. Time was treated as a nominal variable. The adjustment for baseline values was done to address regression to the mean effect. To deal with interdependency and unequal variances due to the repeated measurements design, the R matrix was specified with Time and Player ID as blocks, with an unstructured covariance structure, using the REPEATED statement in the MIXED procedure in SAS. Some models had convergence problems due to low sample size at the 48 h time point. In such cases, the 48 h time point was omitted from the models. Results are presented as point estimates with 95 % CI. Statistical significance level was set to $p < 0.05$. Standardized effect sizes (ES; effects divided by the SD of the baseline value) were utilized to indicate the magnitude of the effects. With the creatine kinase, myoglobin, CMJ, and muscle soreness measures, baseline values for all players in

study II were used to provide a more robust standardization. Pearson correlation coefficient was applied to assess relationships between fold changes from pre-match, in the different outcome variables, at the same time point (1, 24, 48 and 72 h) post-match.

3.3.4 Statistical considerations

Paper I and Paper II have utilized MBI (Hopkins et al., 2009), a method for statistical inferences that in addition to point estimates and standardized effect sizes with CI provides "qualitative assessment of chances of clear outcomes". The purpose is to give the reader a practical interpretation on how a result could be implemented, as well as avoiding the negative consequences of the over-emphasis on p-values in science that have ended in a calling for p-values to be retired (Amrhein et al., 2019).

MBI has been massively utilized in sport science publications (Lohse et al., 2020), but in the period of publication of the first two papers, MBI have come under hard criticism from a series of publications (Curran-Everett, 2018; Lohse et al., 2020; Sainani, 2018; Sainani et al., 2019). The main criticisms are centered around high type I error rates when interpreting *possible*- and *likely* substantial effects as real effects, then lowering the standards of evidence. Furthermore, MBI interprets CI incorrectly as bayesian credible intervals, without being bayesian. This happens when assigning probabilities to the interpretation of a traditional confidence interval and making a probabilistic estimate that some true effect was harmful, trivial, or beneficial. The criticism lead to MBI not being accepted from some scientific journals (MSSE, 2023). As a consequence, Paper III was published using traditional statistical significance testing, including p-values. Regardless of using MBI or traditional significance testing, the focus of the interpretations in this thesis and papers have been on point estimates with CI and effect sizes.

Chapter 4

Results

4.1 The sRPE-TL Response to External Load (Paper I)

An extensive summary of the training load from the 18 sessions in study I is presented in table 4.2, grouped by observations, players and sessions. Our model of the data showed that a 2 SD change in the external load variables from the low intensity-threshold variables, had a 100–106 %, very large within-player effect on sRPE-TL (table 4.1). Around this mean effect, we observed large to very large (very likely substantial) variability, hence individual responses in sRPE-TL to PlayerLoad^{TM}, PlayerLoad2D^{TM}, HSRD, and VHSRD (table 4.3 and figure 4.1). As the difference between players with an average low and high external load, we observed 18–20 %, moderate (likely to very likely substantial) between-player effects of PlayerLoad^{TM}, total distance, HSRD, and VHSRD on sRPE-TL (table 4.1 and figure 4.2).

After adjusting for the external load and differences between players, a 21–29 %, large (most likely substantial) between-session variability was observed in the models with the low intensity-threshold variables. A higher, 35–54 %, very

Table 4.1: The within-player and between-player effect of the specific external load variable on sRPE-TL. The effect is gauged by 2 standard deviations (SD) of the external load variable.

External load variable	Value of 2SD	Effect (%)	90% CI	ES
Within-player effect				
PlayerLoad^{TM} (AU)	224	106.4	83.3 to 132.5	2.60 ***
PlayerLoad2D^{TM} (AU)	130	102.0	79.8 to 127.1	2.52 ***
Total distance (m)	2011	100.6	82.5 to 20.5	2.68 ***
ln(HSRD)[a]	1.48	47.4	30.3 to 6.6	1.40 ***
ln(VHSRD)[a]	2.58	39.5	20.7 to 1.2	1.18 ***
HIE>1.5 (n)	346	100.3	77.6 to 25.7	2.37 ***
HIE>2.5 (n)	100	75.0	55.7 to 6.7	1.92 ***
HIE>3.5 (n)	33.7	52.4	37.4 to 8.9	1.39 ***
Between-player effect				
PlayerLoad^{TM} (AU)	122	19.4	3.2 to 38.1	0.64 *
PlayerLoad2D^{TM} (AU)	62	16.1	0.6 to 34.0	0.54 *
Total distance (m)	820	17.5	2.7 to 34.5	0.62 *
ln(HSRD)[a]	0.72	19.8	5.9 to 35.7	0.65 **
ln(VHSRD)[a]	1.39	19.3	5.5 to 34.9	0.63 *
HIE>1.5 (n)	168	12.4	−2.1 to 29.1	0.40 *
HIE>2.5 (n)	66	3.9	−9.2 to 19.0	0.13
HIE>3.5 (n)	27	0.1	−13.2 to 15.5	0.00

Abbreviations: AU, arbitrary unit; CI, confidence interval; ES, effect size; HIE, high-intensity events; HSRD, high-speed running distance; sRPE-TL, sRPE training load; VHSRD, very high-speed running distance. Note: Uncertainty is indicated by 90% CI. The effect is gauged by 2 SDs of the external load variable. [a]Natural log transformation. The likelihoods of a clear outcome are: *likely, **very likely, and ***most likely.

Table 4.2: Summary statistics of the training load variables grouped by all observations, mean of all players and mean all of sessions.

Group	sRPE (AU)	Duration (min)	sRPE-TL (AU)	Player-Load™ (AU)	Player-Load2D™ (AU)	Total-distance (m)	HSRD (m)	VHSRD (m)	HIE>1.5 (n)	HIE>2.5 (n)	HIE>3.5 (n)
All observations											
Mean	4.1	75.2	322	467	274	4293	388	108	616	164	47
SD	1.6	13.1	159	125	71	1089	246	133	190	59	21
Min	1.0	38.0	45	174	104	1580	3	1	179	32	5
Max	9.0	98.0	855	872	518	7345	1253	779	1441	425	137
Mean of players											
Mean	4.1	75.2	20	3469	3274	4284	393	108	621	165	47
SD	0.7	2.5	2	661	631	410	108	55	84	33	3
Min	2.6	70.0	91	1334	1201	3752	244	54	520	123	28
Max	5.4	78.6	42	4563	4318	5184	578	210	836	241	78
Mean of sessions											
Mean	4.2	75.4	325	469	275	4305	391	111	618	163	46
SD	1.2	11.4	126	99	57	903	181	101	152	43	14
Min	2.0	45.2	96	217	134	1813	162	21	306	72	17
Max	6.8	93.7	618	652	383	5720	853	439	898	258	81

Abbreviations: AU, arbitrary unit; HIE, high-intensity events; HSRD, high-speed running distance;
sRPE, session rating of perceived exertion; sRPE-TL, sRPE training load; VHSRD, very high-speed running distance.

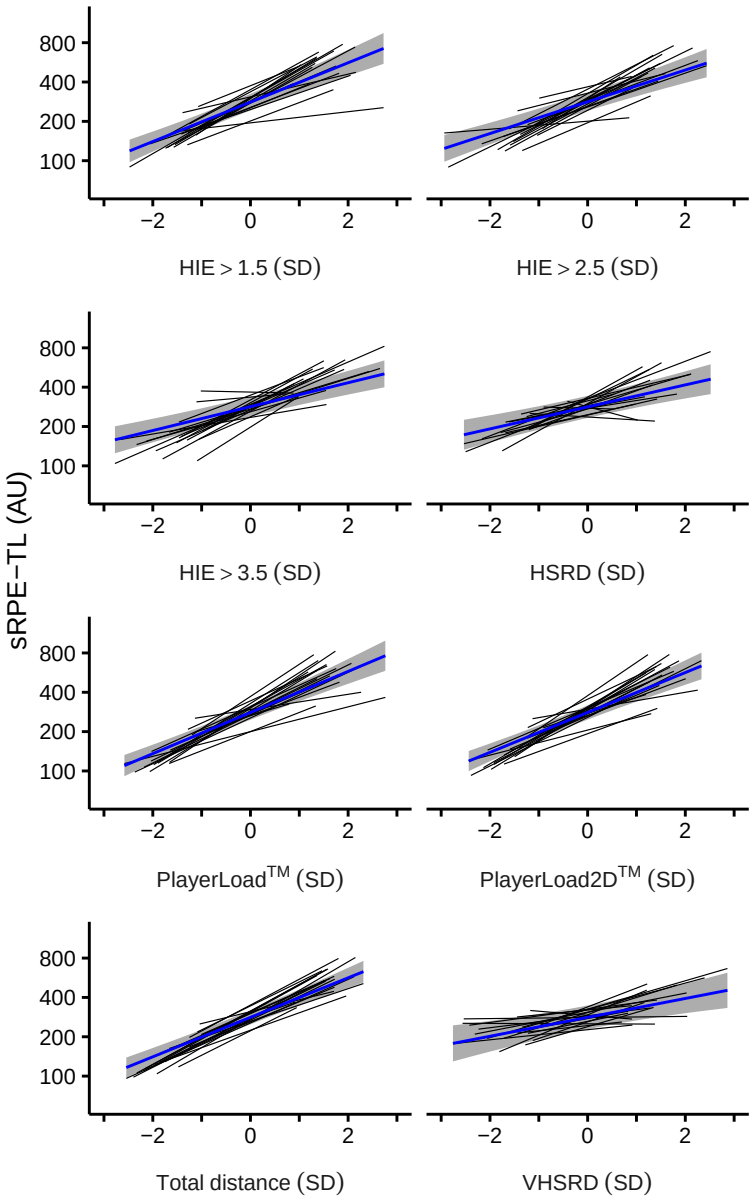


Figure 4.1: sRPE-TL predicted by external load highlighting individual response (thin lines), for all external load variables.The solid regression lines are the within-player effect with 95 % confidence intervals (shaded area). The x-axis shows the external load in number of SDs relative to the individual players' mean external load. The y-axis on all panels has a logarithmic scale. HIE indicates high-intensity events; HSRD, high-speed running distance; sRPE-TL, session rating of perceived exertion training load; VHSRD, very high-speed running distance

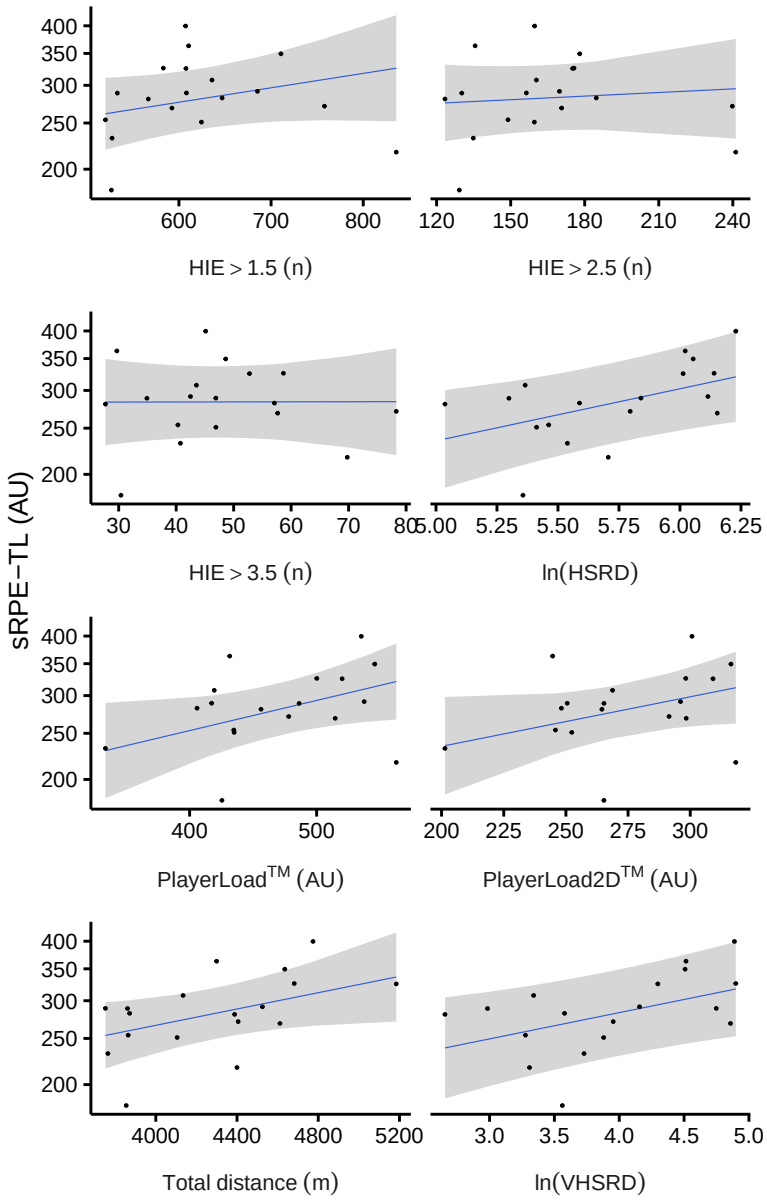


Figure 4.2: The individual players' sRPE-TL predicted by their mean external load value, for all external load variables. The regression line is the between-player effect of external load variables on sRPE-TL, with 95 % confidence intervals (shaded area). The y-axis on all panels has a logarithmic scale. HSRD and VHSRD are transformed to their natural log. HIE indicates high-intensity events; HSRD, high-speed running distance; sRPE-TL, session rating of perceived exertion training load; VHSRD, very high-speed running distance.

large (most likely substantial) between-session variability was observed in the models with the high intensity-threshold variables. Finally, sRPE-TL showed a within-player CV of 23 % (90 % CI; 21 to 26 %) in a typical session, when adjusted for either PlayerLoad^{TM}, PlayerLoad2D^{TM}, or total distance (table 4.3).

Table 4.3: Random Effects Describing the Variability in sRPE-TL That Is Not Explained by the Specific External Load Variable

External load variable	Between-player Variability CV% (90% CI)	Within-player Variability CV% (90% CI)	Between-session Variability CV% (90% CI)	ES	Individual response Variability CV% (90 % CI)	ES
Without predictor	14 (5 to 19)	35 (32 to 40)	55 (34 to 73)			
PlayerLoad^{TM}	20 (11 to 27)	23 (21 to 26)	21 (13 to 27)	0.68***	24 (10 to 33)	0.76**
PlayerLoad2D^{TM}	20 (11 to 26)	23 (21 to 26)	21 (13 to 28)	0.69***	23 (9 to 32)	0.73**
Total distance	16 (9 to 22)	23 (21 to 26)	24 (14 to 31)	0.81***	13 (-7 to 21)	0.47
HSRD	13 (6 to 18)	28 (25 to 31)	49 (31 to 65)	1.45***	22 (5 to 32)	0.72**
VHSRD	13 (5 to 18)	28 (26 to 32)	54 (34 to 72)	1.55***	30 (5 to 44)	0.93**
HIE>1.5	19 (10 to 26)	26 (23 to 29)	29 (18 to 38)	0.87***	19 (3 to 28)	0.59*
HIE>2.5	17 (8 to 23)	27 (25 to 31)	35 (22 to 46)	1.04***	17 (-8 to 27)	0.55
HIE>3.5	16 (7 to 22)	30 (27 to 34)	40 (25 to 53)	1.12***	11 (-9 to 19)	0.34

Abbreviations: CI, confidence interval; CV, coefficient of variation; ES, effect size; HIE, high-intensity events; HSRD, high-speed running distance; sRPE-TL, session rating of perceived exertion training load; VHSRD, very high-speed running distance. Note: Thresholds for ESs for random effects are: >0.1, small; >0.3, moderate; >0.6, large; >1.2, very large; and >2.0, extremely large. The random effects are separated into within-player, between-player, and between-session variability, and individual response to external load, all presented as CV% with 90% CIs. Between-session variability and individual response are evaluated by magnitude-based inferences. The likelihoods of a clear outcome are: *likely, **very likely, and ***most likely.

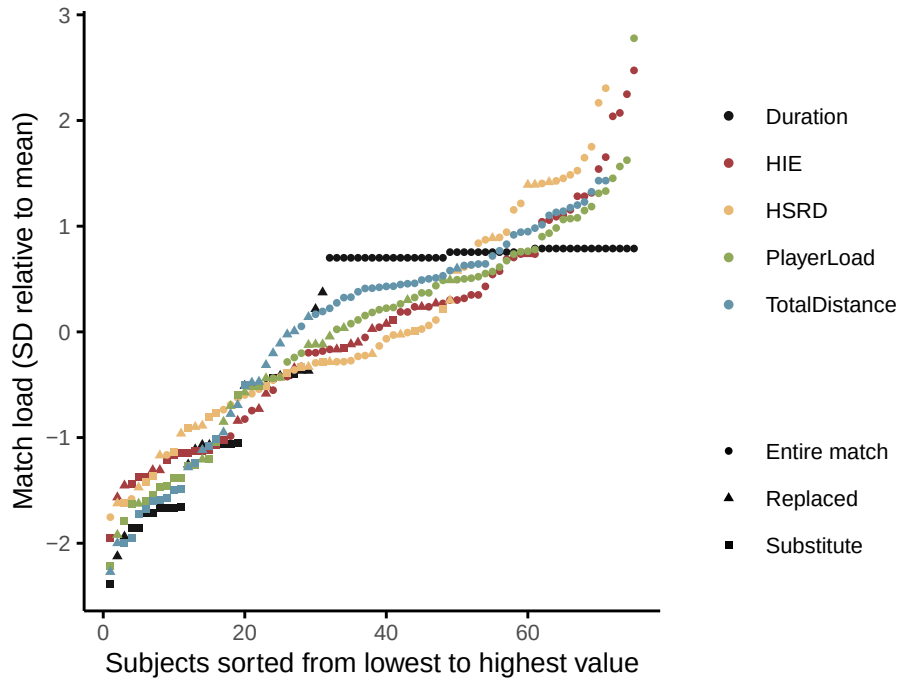


Figure 4.3: Each external load variable sorted from the player with the lowest to the player with the highest value to indicate the spread of the match load

4.2 The effect of external load on recovery markers after a football match (Paper II)

The results from study II is presented in the following sections.

4.2.1 Summary of the match load

The final scores from the three matches were 2–1, 2–1 and 6–3. As a result from the pre-planned substitutions and the variability between players, the match load across all players was spread in a linear manner for all external load variables (figure 4.3). The only exception was the duration where 61 % of the players played a full 90 min match. A descriptive summary of total and relative match load is shown in table 4.4.

Table 4.4: Summary of total match load and match load per minute for selected external load variables, for all players and for different groups of players (mean ± SD).

Group	**n**	**Duration (min)**	**Distance (m)**	**PlayerLoad^{TM} (AU)**	**HIE>2.5 (#)**	**HSRD (m)**
Total match load						
All	75	72.7 ± 24.9	8,305 ± 2,627	780 ± 290	152 ± 62	380 ± 200
Entire match	44	91.2 ± 1.0	10,110 ± 972	966 ± 174	185 ± 52	434 ± 199
Replaced	16	54.7 ± 16.8	6,673 ± 2,016	637 ± 191	124 ± 43	357 ± 205
Substitute	15	37.4 ± 13.7	4,483 ± 1,075	386 ± 123	85 ± 31	237 ± 113
Match load per min						
All	75		116 ± 14	10.8 ± 1.8	2.2 ± 0.6	5.4 ± 2.6
Attackers	10		112 ± 7	10.0 ± 1.2	2.1 ± 0.4	5.0 ± 2.3
Central defenders	14		101 ± 5	9.1 ± 1.0	1.7 ± 0.5	3.2 ± 1.3
Central midfielders	22		128 ± 12	12.2 ± 1.9	2.4 ± 0.5	5.0 ± 2.3
Fullbacks	13		112 ± 14	10.6 ± 1.0	2.0 ± 0.5	6.0 ± 1.6
Wide midfielders	16		117 ± 12	11.3 ± 1.5	2.4 ± 0.5	8.0 ± 2.7
Entire match	44		111 ± 11	10.6 ± 1.9	2.0 ± 0.6	4.8 ± 2.2
Replaced	16		123 ± 11	11.8 ± 1.6	2.3 ± 0.4	6.4 ± 2.8
Substitute	15		123 ± 21	10.5 ± 1.4	2.4 ± 0.6	6.5 ± 3.2

Table 4.5: Summary of baseline values for recovery markers and performance tests in study II.

Variable	n	Mean	SD	Min	Max
CK (U/L)	49	367	273	59	1600
MYO (μ/L)	49	39	37	21	256
CMJ (cm)	59	43.0	4.5	33.2	57.5
SP30 (s)	32	4.27	0.18	3.62	4.53
YOYO (m)	24	2000	188	1299	2800

4.2.2 Mean Change in Recovery Markers

Baseline values of the recovery markers are shown in table 4.5, and the mean changes in recovery markers from pre- to 1, 24, 48 and 72 h post-match are presented in figure 4.4. The matches induced most likely substantial increases in CK at 1 h (ES = 0.92), 24 h (ES = 1.20), and 48 h (ES = 0.67) post-match, whereas a likely substantial increase was seen 72 h post-match (ES = 0.32). Myoglobin peaked at 1 h post-match with a most likely substantial increase (ES = 3.80), followed by a most likely substantial increase at 24 h (ES = 0.78), and possibly substantial increases at 48 h (ES = 0.27) and 72 h (ES = 0.30). CMJ height showed a most likely substantial decrease at 1, 24 and 48 h and a likely substantial decrease at 72 h post-match with ES of −0.75, −0.68, −0.68 and −0.25 respectively. SP30 showed a likely substantial increase (ES = 0.38) at 72 h post-match, while for YOYO, the effect was trivial and unlikely substantially positive (ES = −0.08).

4.2.3 The Effects of External Load Variables on Recovery Markers

The effects of the external load variables on recovery markers at each time-point are presented in figure 4.5 and 4.6. The external load variables had positive effects on the muscle damage indicators – a higher load was associated with higher levels of the blood markers. HSRD had the strongest relationship with CK showing very likely to most likely substantial effects, consistent throughout all time-points (ES = 0.60–1.08). Duration, total distance, and HIE showed likely substantial effects on CK at 1 h (ES = 0.33–0.42), 24 h (ES = 0.44–0.50), and 72 h (ES = 0.49–0.66). The effects on myoglobin at 1 h post-match was very likely substantial for HSRD (ES = 0.80) and likely substantial for duration (ES = 0.65), HIE (ES = 0.68), total distance (ES = 0.58), and PlayerLoad^{TM} (ES = 0.49). Except for a likely substantial increase of HSRD (ES = 0.49) and a possibly substantial effect of Duration (ES = 0.31) at 24 h, the other effects at 24 and 48 h post-match were unclear. At 72 h, likely substantial effects on myoglobin were found for all variables (ES = 0.52–0.69). The observed effects on CMJ were generally trivial or unclear, except for a possibly substantial negative effect of HIE at 24 h (ES = −0.26) and a likely substantially positive effect of HSRD at 48 h post-match (ES = 0.40). SP30 performance 72 h post-match was affected negatively by total distance (ES = 0.56) and PlayerLoad^{TM} (ES = 0.46), showing likely substantially negative effects. On the contrary, likely substantially

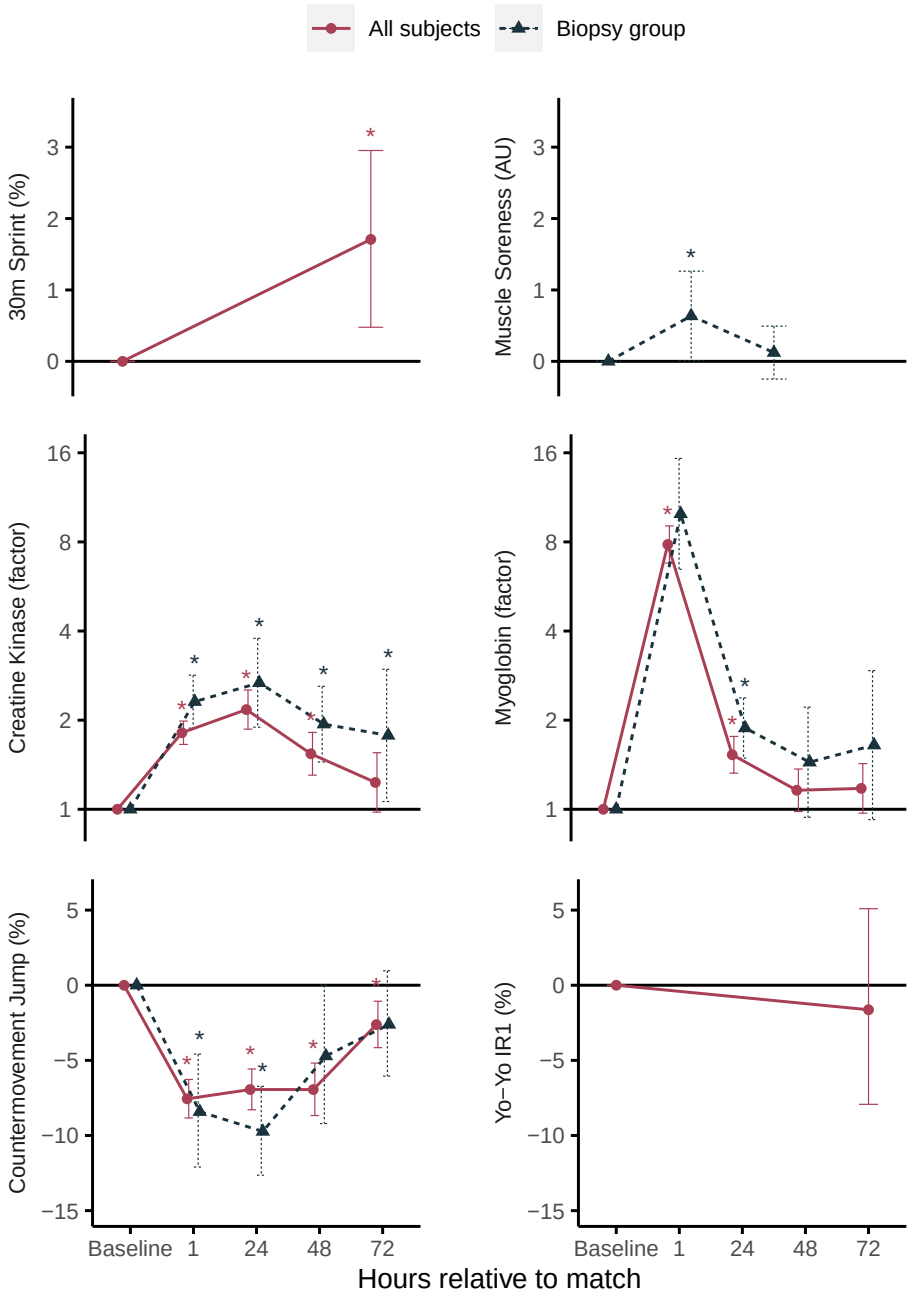


Figure 4.4: Estimated change from baseline for the specific recovery markers in the hours post-match for all subjects, and for the subgroup with muscle biopsies. Estimates are adjusted for baseline (both groups), and for PlayerLoad^{TM} and YOYOmax (all subjects). Error bars indicates 95 % CI. * = $p < 0.05$.

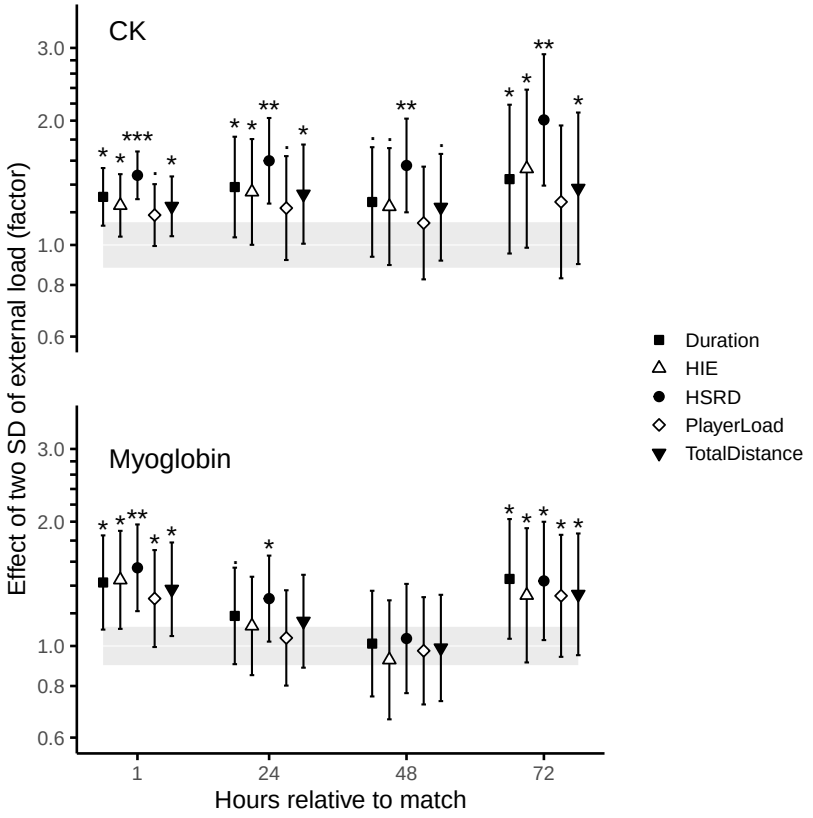


Figure 4.5: The factor effect of two SDs of match load on recovery markers at the specific time-points, adjusted for baseline and YOYOmax. Two SDs of match load are interpreted as the difference between matches with typical high and low load. Uncertainty in the estimates is indicated by 90 % confidence intervals and shaded area represents trivial changes. Probabilistic statements about the true effect are labeled as follows: · = possibly, * = likely, ** = very likely, and *** = most likely.

positive effects of HIE (ES = 0.56) and PlayerLoad^{TM} (ES = 0.47) were seen on YOYO performance 72 h post-match.

4.2.4 Effect of External Load Variables on Recovery Status 72 h Post-match

The predicted mean changes in recovery markers at 72 h for given match loads are depicted in figure 5.3. External load variables that are substantially affecting recovery markers are highlighted in figure 4.5 and 4.6. Other external load variables were non-substantial meaning that a change in match load could cause either trivial change, or substantial increase or decrease in the recovery markers. While substantial effects were seen on predicted means for some external load variables, prediction intervals for individual values covered both substantially negative and substantially positive values throughout the range of match load

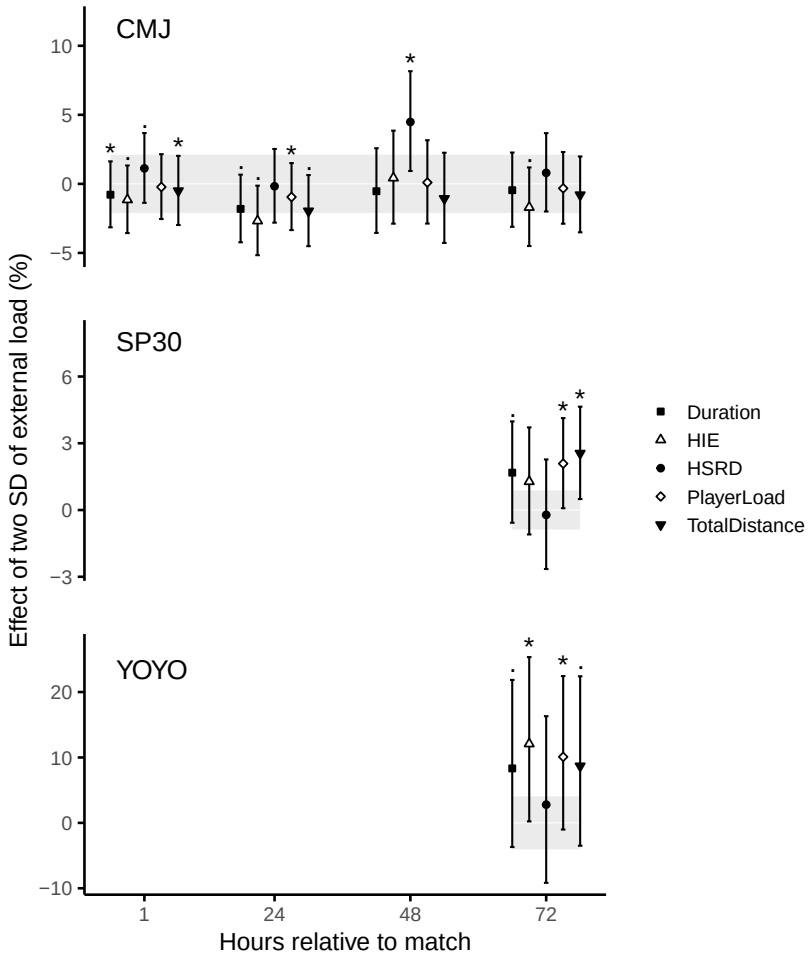


Figure 4.6: The effect of two SDs of match load on recovery markers at the specific time-points, adjusted for baseline and YOYOmax. Two SDs of match load are interpreted as the difference between matches with typical high and low load. Uncertainty in the estimates is indicated by 90 % confidence intervals and shaded area represents trivial changes. Probabilistic statements about the true effect are labeled as follows: · = possibly, * = likely, ** = very likely, and *** = most likely.

on all external load variables.

4.3 Muscle damage and Heat Shock Proteins response to a football match (Paper III)

In Paper III, a subset of 12 players from study II donated muscle biopsies in addition to the blood samples and recovery tests outlined in Paper II.

4.3.1 Match Load, neuromuscular fatigue, and muscle soreness

The players' performed on average a total distance covered of $10\,114 \pm 1002$ m, a high-speed running distance of 492 ± 195 m, and a PlayerLoad^{TM} of 990 ± 147 AU. One player was substituted after 68 min due to knee pain. All other players played a full 90 min match. The mean CMJ height at baseline was 42.5 ± 2.3 cm. Decreases in CMJ height of $-8.4\,\%$ (95 % CI; -12.1 to $-4.6\,\%$, ES $= -0.85$, $p < 0.01$) at 1 h, $-9.7\,\%$ (CI; -12.6 to -6.7 , ES $= -0.98$, $p < 0.01$) at 24 h, $-4.7\,\%$ (CI; -9.2 to 0.0 , ES $= -0.46$, $p = 0.05$) at 48 h, and $-2.6\,\%$ (CI; -6.0 to 1.0 , ES $= -0.25$, $p = 0.13$) at 72 h were observed (figure 4.4). On a 5-point scale, muscle soreness increased by 0.68 units (CI; 0.01 to 1.3, ES $= 1.00$, $p = 0.047$) at 24h and then decreased toward baseline level at 48 h (figure 4.4).

4.3.2 Muscle damage indicators in blood

At baseline, the mean creatine kinase value was 367 ± 225 U/L, increasing 2.31-fold (CI; 1.88 to 2.84, ES $= 1.34$, $p < 0.01$) at 1 h and peaking 2.67-fold (CI; 1.89 to 3.78, ES $= 1.60$, $p < 0.01$) at 24 h. At 72 h post-match, there was still a 1.77-fold increase (CI; 1.06 to 2.97, ES $= 1.00$, $p = 0.033$) compared to baseline (figure 4.4). Myoglobin was $32 \pm 10\,\mu g\,L^{-1}$ at baseline and peaked at 1 h with a 9.95-fold increase (CI; 6.43 to 15.23, ES $= 4.12$, $p < 0.01$), returning to a 1.88-fold increase (CI; 1.49 to 2.38, ES $= 1.06$, $p < 0.01$) at 24 h compared to baseline. At 48 and 72 h, factor increases of 1.44 and 1.65 were observed, respectively, but with larger uncertainty (figure 4.4).

4.3.3 Western blot analyses of HSPs

In the cytosolic fraction, αB-crystallin decreased by a factor of 0.83 (CI; 0.75 to 0.92, ES $= -0.73$, $p < 0.01$; figures 4.7 and 4.8) at 1 h and returned to baseline values at 24 h post-match. A secondary decrease in αB-crystallin was observed 48 h after the match, however with a large uncertainty (CI; 0.65 to 1.10). In the cytoskeletal fraction, a 3.63-fold increase (CI; 1.98 to 6.66, ES $=$ 4.94, $p < 0.01$) was observed 1 h post-match and αB-crystallin levels remained high in the cytoskeletal fraction until returning toward baseline level at 72 h. Cytosolic levels of HSP70 decreased by a factor of 0.85 from baseline (CI; 0.76 to 0.95, ES $= -0.78$, $p = 0.001$) at 1 h post-match. HSP70 levels were still lower by a factor of 0.92–0.93 (CI; 0.73 to 1.18) at 24 and 48 h and 0.83 (CI; 0.68 to 1.02, ES $= -0.89$, $p = 0.072$) at 72 h post-match compared to baseline, but with

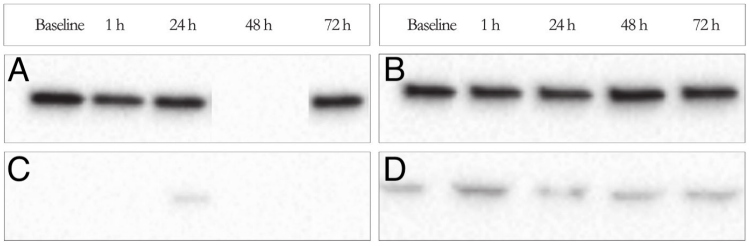


Figure 4.7: Western blot bands for αB-crystallin, showing cytosol levels (A) and cytoskeleton levels (C). HSP70 bands show cytosol levels (B) and cytoskeleton levels (D). The missing band in the 48 h αB-crystallin lane (A) was due to this subject missing the 48 h biopsy

greater uncertainty and therefore statistically non-significant. In the cytoskeletal fraction, HSP70 levels increased 1.78-fold (CI; 1.26 to 2.49, ES = 2.79, $p < 0.01$) at 1 h and remained approximately at the same levels until 72 h. The increase in the cytoskeletal fraction observed at 1 h corresponded to roughly 10 % the total cytosolic plus cytoskeletal levels at baseline.

4.3.4 Immunohistochemistry analyses of HSPs and Tenascin-C

Analysis of muscle fiber types revealed that the players had a larger proportion of type II fibers (66 % CI; 60 to 71) compared to type I fibers. Staining intensity of both αB-crystallin and HSP70, in each fiber type, showed similar patterns with an increase from baseline at 24 h, a peak at 48 h and a reduction to approximately the 24 h levels at 72 h (figure 4.9). Specifically, αB-crystallin increased by 22 % (CI; 7 to 39, ES = 0.82, $p < 0.01$) in the type I fibers and 27 % (CI; 11 to 46, ES = 1.05, $p < 0.01$) in the type II fibers at 24 h and was still significantly elevated at 72 h. HSP70 showed a 20 % increase (CI; 6 to 36, ES = 0.93, $p < 0.01$) in type I fibers and a 13 % (CI; -0.1 to 27, ES = 0.76, $p = 0.052$) in the type II fibers at 24 h, however, at the other time points the confidence intervals also covered negative values (figure 4.9). While αB-crystallin staining intensity peaked at 48 h (both fiber types), it is unknown whether HSP70 peaked at 24 h or 48 h due to the missing time point at 48 h.

Granular staining of αB-crystallin was observed in 1.0 ± 0.7 h of the fibers at baseline. The proportion of fibers with granular staining increased at 1 h by a factor of 2.2 (CI; 1.3 to 3.6, ES = 1.21, $p < 0.01$) and 1.6 at 24 h (CI; 1.0 to 2.5, ES = 0.71, $p = 0.046$). At 72 h, the proportion of granular stained fibers returned to baseline level, although with large uncertainty (figures 4.10 and 5.4). Granular staining was observed in both fiber types. At baseline, 0.93 ± 0.52 % of the analyzed area showed immunoreactive tenascin-C. The observed average stained area increased by factors of 1.56, 1.15 and 1.20 at 1, 24 and 72 h, respectively, with great uncertainty, but all effects were statistically non-significant ($p > 0.05$; figures 4.10 and 5.4).

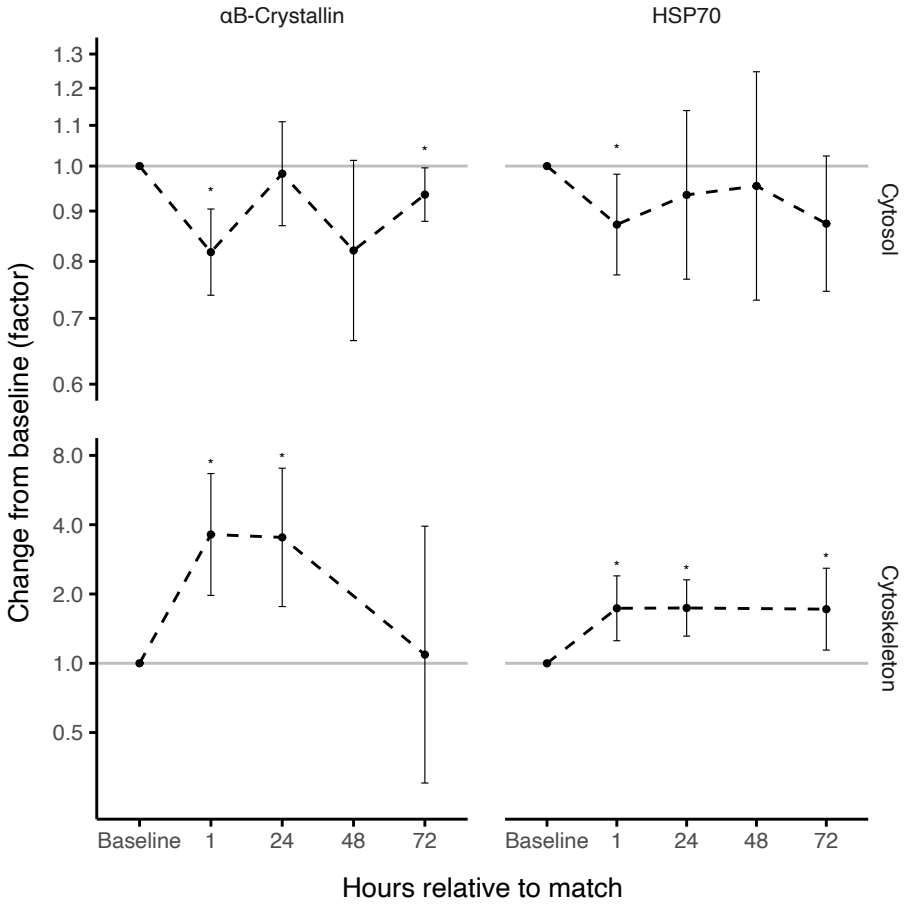


Figure 4.8: αB-crystallin and HSP70 measured by Western blotting as factor change from baseline, in the period after the match. Upper panes show the cytosol fraction, whereas the lower panes show the cytoskeletal fraction. Uncertainty in the estimates is indicated with 95 % CI. * different from baseline values ($p < 0.05$)

4.3.5 Correlations

No statistically significant correlations were observed between change in any HSP measures and change in CMJ, CK, or myoglobin after the match. Change in staining intensity levels were correlated in type I and type II fibers in αB-crystallin ($r = 0.88$, $p < 0.01$) and HSP70 (r = 0.96, $p < 0.01$) at 1 h post-match. Furthermore, changes in cytoskeletal αB-crystallin levels were correlated with changes in αB-crystallin staining intensity in type I ($r = 0.74$, $p < 0.01$) and type II ($r = 0.74$, $p < 0.01$) fibers at 1 h post-match. For HSP70, however, correlation effects were trivial. Changes in creatine kinase and myoglobin were strongly correlated (r =0.92–0.98, $p < 0.01$) across all time points.

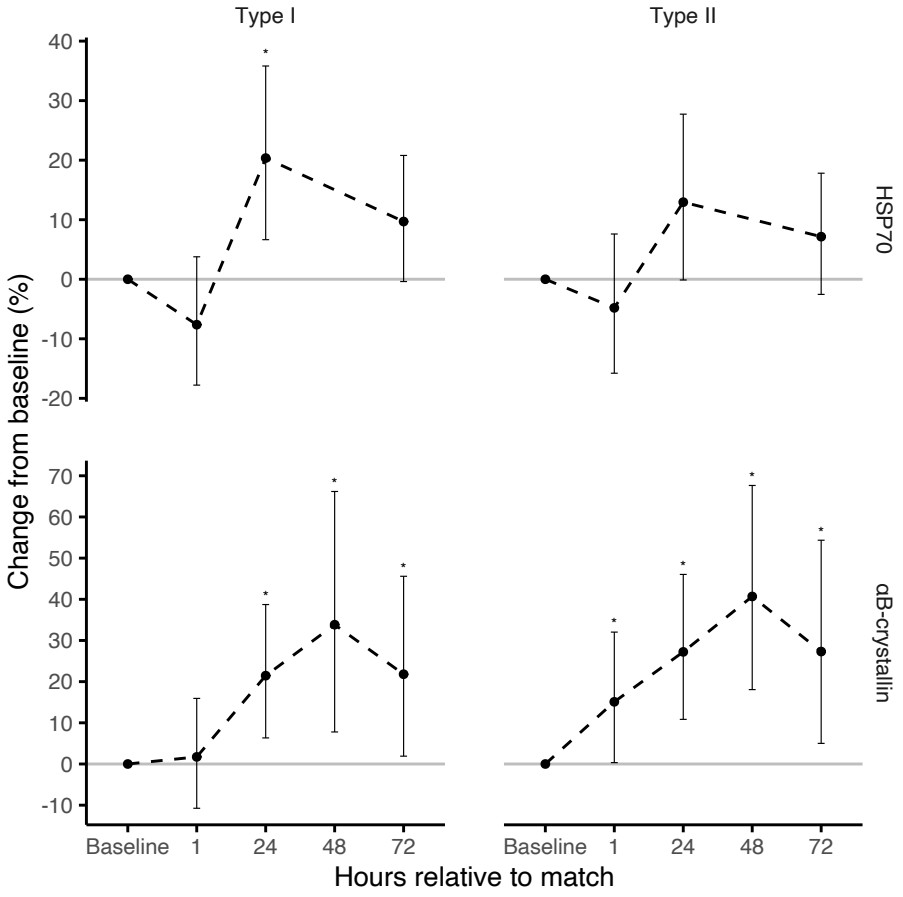


Figure 4.9: Percent change in staining intensity from baseline for αB-crystallin and HSP70, measured by immunohistochemistry, in the period after the match. Left panes show HSP response in type I muscle fibers, whereas right panes show HSP response in type II muscle fibers. Uncertainty in the estimates is indicated with 95 h CI. * different from baseline values ($p < 0.05$)

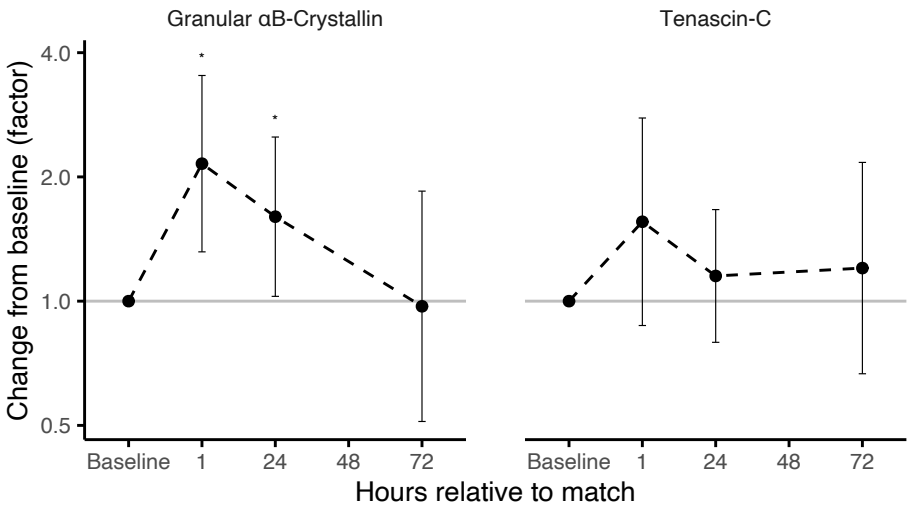


Figure 4.10: Factor change from baseline to specific time point post-match for granular staining of αB-crystallin and for tenascin-C. Uncertainty in the estimates is indicated with 95 % CI. * different from baseline values ($p < 0.05$)

Chapter 5

Discussion

Quantifying training- and match load, both internal and external load, and the recovery of blood markers and muscle function after football matches have been extensively researched previously. The novelty in this thesis is, however, in three main areas. Firstly, in modelling the individual response to external load, and the variability of the sRPE-TL method. Secondly, the effect of external load on recovery markers after a football match, and third, the research on muscle cellular damage, via HSP as proxy markers. The following section provides a discussion of the three main areas.

5.1 Effects of external load on internal load

In Paper I, we modeled the effect of external load variables on sRPE-TL during training sessions in elite football players, using an individual approach. We found that external load variables with low intensity-thresholds were closely related to sRPE-TL; however, the relationship became weaker with increasing intensity thresholds. Furthermore, small to moderate between-player effects of external load were evident for most of the external load variables. Finally, the data show moderate to large individual responses to PlayerLoad^{TM}, PlayerLoad2D^{TM}, HIE > 1.5, HSRD, and VHSRD. Although external load had large to very large within-player effects on sRPE-TL, there was still large to very large between-session variability in sRPE-TL, as well as between-player variability and within-player variability that could not be explained by external load variables.

Our results show that sRPE-TL could differentiate 2 SD of the external load variable, corresponding to the difference between a typical low- and high-load session (large to very large effect size). In fact, even 0.5 SD change in PlayerLoad^{TM} and total distance led to an approximate 20 % difference in sRPE-TL (moderate, very likely to most likely substantial effects) from these variables (data not shown). The ability of sRPE-TL to discriminate between different amounts of external load within the same player suggests that sRPE-TL is a valid tool quantifying training load, in accordance with existing literature (Foster et al., 2001; Impellizzeri et al., 2004; McLaren et al., 2018).

While the ability of sRPE-TL to differentiate between varying amounts of external load within the same player underscores its utility in quantifying internal training load, it's important to acknowledge that the *true* load encompasses several components and is a complex concept. One potential limitation of sRPE-TL, and similar methods, is their inability to accurately discern how training load impacts specific muscles or structures. This raises questions about the challenges associated with using more global measurements versus understanding load-stress on more isolated muscles and structures. Further exploration in this

area could provide valuable insights into the nuanced dynamics of training load management.

5.1.1 The relationship between sRPE-TL and external load variables

Session rating of perceived exertion training load had the strongest relationship with the external load variables with no threshold or low intensity-thresholds, that is, PlayerLoad^{TM}, PlayerLoad2D^{TM}, total distance, and HIE > 1.5 (table 4.1), in agreement with other studies on team sports (Casamichana et al., 2013; Gallo et al., 2015; Lovell et al., 2013; McLaren et al., 2018; B. R. Scott et al., 2013; T. J. Scott et al., 2013). This finding suggests that the sRPE-TL first and foremost reflects the total work completed, rather than periods of high-intensity work. This could be attributed to the fact that sRPE-TL and the low intensity-threshold variables are strongly related to the session duration as they account for all work irrespective of its intensity. In contrast, the high intensity-threshold variables are more related to the duration of high intensity work, rather than the total duration of sessions. The external load variables with high intensity-thresholds (HSRD, VHSRD, HIE > 2.5, and HIE > 3.5) showed weaker relationships with sRPE-TL, although still large, most likely substantial effects were evident. This weaker relationship might partly be due to the reduced precision of global navigation satellite systems at higher speeds, (E. Rampinini et al. in 2014). However, a more likely explanation is that high-intensity activities constitute a minor portion of total work in a typical training session, which then affects how it relates to sRPE-TL. In fact, many sessions had very little high-intensity work at all (table 4.2). In addition, in some players these variables had a negative effect on sRPE-TL (figure 4.1). Interestingly, compared with a reference model without any external load predictors, these variables explained very little of the between-session variability and also less of the within-player variability in sRPE-TL than the low intensity-threshold variables (table 4.3). From these perspectives, variables describing high-intensity work is not only inferior, but also unsuitable as single predictors of training load, particularly when training regimes include low-intensity training sessions. Nonetheless, this should not undermine the importance of high-intensity work in training load. High-intensity efforts are undeniably strenuous, and high-intensity external load variables have successfully been used in multiple regression to predict sRPE-TL (Gaudino et al., 2015) and in machine learning models predicting pure RPE (Bartlett et al., 2017; Jaspers et al., 2018). More studies are indeed needed, especially on predicting sRPE-TL using machine learning techniques.

5.1.2 Between-player effects; players with different average external load

The between-player effect describes the difference in sRPE-TL between players with a typically low and typically high *mean* external load. The variables based on PlayerLoad^{TM} and distance showed borderline small to moderate, likely

substantial effects, indicating that players who do more external training load on average likely report higher sRPE-TL. Consequently, if a group of players perform external load on their own average, it will not result in the same sRPE-TL for everyone. This means that normalizing the external load will not be adequate method to individualize external load to account for differences in internal load. It also means that external load alone is probably not sufficient when monitoring individual training load. A possible interpretation is that players with higher average external loads might be exerting more effort, hence reporting elevated sRPE-TL compared to those with lower average loads, aligning with the intended function of sRPE-TL. The between-player effect could be skewed if some players consistently participated in sessions with either high or low training loads, however, this potential skewness is addressed in the statistical analysis through a random intercept for session ID. Although small to moderate between-player effects of external load were found, there was still 13–20 % between-player *variability* in sRPE-TL that could not be explained by the external load variables (table 4.3), reflected by the wide CIs in figure 4.2. It is also worth noting that the CI for the PlayerLoad^{TM} and total distance effects are wide and the data also is compatible with trivial effects, although not substantially negative effects. No substantial between-player effects were found for HIE > 2.5 and HIE > 3.5.

5.1.3 Individual response to external load

An important finding in this study was the individual response in sRPE-TL to external load, represented as individual slopes in figure 4.1 and with individual data in figure 5.1. Two SDs of PlayerLoad^{TM}, PlayerLoad2D^{TM}, HSRD, and VHSRD resulted in large (very likely substantial) variability in sRPE-TL response, whereas HIE > 1.5 resulted in a moderate (likely substantial) variability in sRPE-TL response (table 4.3). In practice, this means that, a 224 AU increase in PlayerLoad^{TM} will lead to 106.4 % increase in sRPE-TL on average, with a $\pm$ 24 % SD for a group of players (table 4.1 and 4.3). This finding supports the theory that internal load is determined by external load in interaction with individual characteristics (Impellizzeri et al., 2005, 2019). The individual differences in sRPE-TL response underlines the importance of individualized monitoring of training load in team sports and the need for monitoring internal load in addition to external load. While the current study did not assess individual characteristics of players, other studies have found that individual characteristics such as experience, position, and time-trial performance functioned as mediators of the relationship between external load and sRPE-TL in Australian rules football (Gallo et al., 2015), and athletes with greater maximal oxygen uptake seem to rate lower sRPE-TL and sRPE (Garcin et al., 2004; Milanez et al., 2011). These studies as well as the current study highlight the individual differences when rating sRPE-TL. Contradictory to our results, Jaspers et al. (2018) found that prediction of pure sRPE from external load variables using machine learning techniques could be made more accurate from models on a group of players, than from models on individual players. The reason for the higher accuracy with the group models could be because of far larger sample size compared

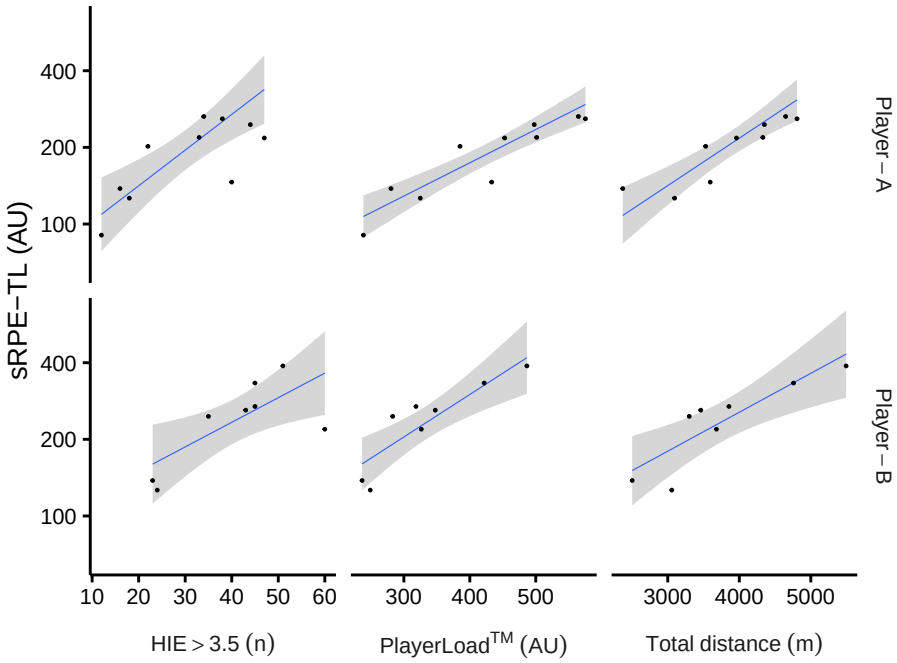


Figure 5.1: Session rating of perceived exertion training load (sRPE-TL) predicted by external load for 3 different external load variables,in 2 different players. Uncertainty is indicated by 90% confidence intervals (shaded area). HIE indicates high-intensity events.

with the models on individuals. In addition, the external load relationships with sRPE could be different than with sRPE-TL. Interestingly, the individual response observed for total distance was lower and unclear. This makes total distance more uniformly related to sRPE-TL across players. If such findings are consistent, and given that total distance has the strongest correlation with sRPE-TL, total distance seems to be the most preferable training load measure when a single measure is used.

5.1.4 Session to session variability and the explanatory power of external load variables

The random effects from the model are estimations of the variability in sRPE-TL that were not explained by the external load variables (table 4.3). The *between-session variability* represents the unexplained variability in sRPE-TL due to that every session is different, i.e. the players rated some sessions higher on average than other sessions, after adjusting for external load in the model. In the models with the strongest external load variable predictors, the between-session variability was ± 21 %. However, it is clear that the between-session variability increases as the intensity-threshold for the external load variables increases. The poor ability to explain the between-session variability of sRPE-

TL for the high intensity-threshold variables, suggests these variables to be unsuitable as single predictors of sRPE-TL when monitoring multiple training sessions. Furthermore, the between-session variability highlights that even the best external load variables fail to cover some of the overall load completed in the different sessions. This could be due to the arbitrary selection of external load variables from tracking devices, or the lack of sensitivity in the tracking devices to measure the overall external load. Hence, valuable information about the load is probably lost when using single external load variables. The sensitivity of external load variables to measure the overall external load may be dependent on training mode. In fact, in the meta-analysis from McLaren et al. (2018), training mode was moderating the relationships between external load variables and sRPE-TL. This is also demonstrated earlier by variability in correlation coefficient between sRPE-TL and external load (Lovell et al., 2013; Weaving et al., 2014) and sRPE-TL and HR (Alexiou & Coutts, 2008) across different training drills. The effect of training mode was not evaluated in this study.

5.1.5 The reliability of sRPE-TL

Despite its widespread use, the reliability of the sRPE method is scarcely researched. In this context, the reliability means how consistent the players will rate sRPE-TL if a specific session was repeated multiple times. In studies with standardized running and cycling protocols at different intensities, sRPE showed poor reliability with CVs of 28–32 % (T. J. Scott et al., 2013; Wallace, Slattery, & Coutts, 2014). The challenge of reproducing the same training load in repeated sessions makes it difficult to assess the reliability of sRPE-TL method from field sessions. In this study, the *within-player variability* (i.e. the residuals from the model) represents the individual players' variability in sRPE-TL in a typical session, after adjusting for the external load, and the session variability (Session ID). Thus, we propose a reasonable estimate of the reliability of sRPE-TL of 23 % (90 % CI, %21–26 %). The validity of this estimation is however dependent on the degree that the external load variable and random effect for session ID adjust adequately and consistently for the differences in true training load between sessions, and that the individual players' characteristics are consistent across the study period. Reasons for the poor reliability of sRPE-TL are for now speculations, however this could include several factors. Some variability could come from the crude 10-point scale of the RPE (Fanchini et al., 2016), allowing players only to rate whole numbers. For example, if a player would like to rate 5.5 RPE instead of 5 RPE after a session, that corresponds to a 10 % difference in sRPE-TL. Furthermore, half a point from 1 and 10 corresponds to a 50 and 5 % difference respectively. On the other hand, given the 24 h or more separation between training sessions, players may find it hard to compare or rank the intensity between sessions and, therefore, not be consistent in the rating of the RPE score. In addition, different recovery status before trainings, change in fitness status during the season, or the ability of the external load variables to reliably explain the true training load undertaken by the players could also affect the reliability. It's important to consider that some of these

factors, such as changes in fitness status during the season, directly impact the internal load experienced by players. This means that variations in sRPE-TL ratings, in response to evolving fitness levels, reflect genuine changes in internal load rather than merely being artifacts of the measurement method. Therefore, the observed variability in sRPE-TL could be indicative of real fluctuations in the players' physiological and psychological responses to training, underscoring the dynamic nature of internal load across a competitive season. Clearly, a comprehensive reliability study is needed to investigate the potential reasons for poor reliability of sRPE-TL.

5.2 Effects of external load on recovery after match

In Paper II we investigated how external load variables, derived from player tracking devices, affected subsequent recovery of CMJ, CK, myoglobin, SP30 and YOYO up to 72 h post match. The external load variables were found to impact both the magnitude and the length of the recovery. HSRD was the strongest predictor of muscle damage indicators, while PlayerLoad^{TM} and total distance predicted recovery of sprint performance, and HIE and PlayerLoad^{TM} predicted YOYO performance. Unexpectedly, recovery of CMJ performance could not be predicted by any of the external load variables. Despite these substantial mean effects, external load variables were not able to predict recovery in individual players.

5.2.1 Recovery of neuromuscular function

We observed a 1.6 %, small effect size, increase in SP30 time 72 h post match. The decreased SP30 performance at 72 h post-match indicates that sprint performance is not recovered 3 days post-match, in line with some studies (Fatouros et al., 2010; Ispirlidis et al., 2008), but not all (Silva et al., 2013). PlayerLoad^{TM} and total distance showed small effects on SP30 at 72 h. To our knowledge, no other studies have examined such relationship. As opposed to muscle damage, which was affected by high-intensity work, SP30 was affected by variables describing match load volume. In line with this finding, it has been proposed that recovery of sprint performance could be linked to the duration of exercise, as basketball and handball have shown shorter recovery times than football (Doeven et al., 2018).

We observed a 8 %, moderate effect size, decrease in CMJ height 1 h post match, which did not return to baseline after 72 h (small effect). The observed decrease in CMJ performance suggests a neuromuscular fatigue comparable to other studies (Nedelec et al., 2014). Unexpectedly, the decrease in CMJ could not be explained by any of the external load variables. This is in contrast to Rowell et al. (2017) where a dose-response relationship was found between low, medium, and high PlayerLoad^{TM} groups and CMJ height 0.5 and 18 h post-match. Moreover, Russell et al. (2016) found moderate correlations between change in peak power output from CMJ and HSRD and sprint distance. Other

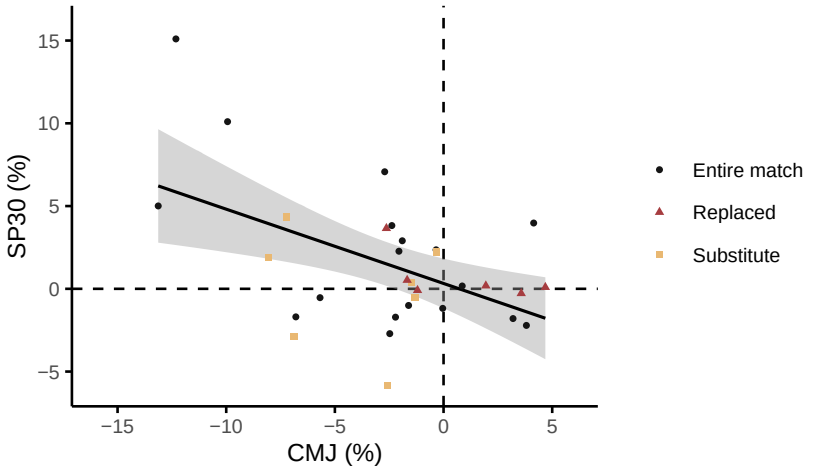


Figure 5.2: Relationship between change in CMJ % and change in SP30 % at 72 h post match ($r = -0.50$, 95 % CI, -0.73 to -0.18, $p = 0.004$). Each data point is one player, and the rotations (substitute, replaced and entire match) are indicated by color and shape of the point.

studies have found short-lived relationships between change in CMJ and high-intensity accelerations (Russell et al., 2016), hard changes of directions (Nedelec et al., 2014) at 24 h, and decelerations at 0.5 and 48 h (de Hoyo et al., 2016). These relationships suggest that CMJ performance could be linked to accelerative efforts that target the same muscles that are active in CMJ. Although we did find a possibly small effect of HIE on CMJ at 24 h, the uncertainty in the estimates and inconsistency over the time-points does not provide strong evidence for such relationship.

What made the lack of relationship between external load surprising was the fact that both CMJ and SP30 performance was impaired 72 h post-match, and that change in SP30 was negatively correlated with change in CMJ at 72 h post-match (figure 5.2, $r = -0.50$). A possible explanation for the lack of relationship could be that just participating in the match, including travel, warm up and playing was enough to cause neuromuscular fatigue, and that the additional variation in external match load was not significant to have an effect. Adding to that, the substitutes and replaced players had higher match load per minute, especially for HSRD (table 4.4). That could have increased the stress on the muscles leading to neuromuscular fatigue, despite the lower total external match load compared to those who played the entire match. A strange finding was the positive effect of HSRD on CMJ after 48 h (small effect), but this is inconsistent both with the theory of "more load - more fatigue" and with the other external load variables and time points, so it may be a spurious effect.

For YOYO, no substantial change was observed from baseline to 72 h post-match. Nevertheless, positive effects of HIE and PlayerLoad^{TM} were still found, suggesting that higher match load improves the YOYO performance 72 h. This finding was unexpected, as one might think that more load leads to reduction

in performance, not the other way around. A possible reason for this could be that a conditioning effect, due to that the match was played a couple of weeks after the season, was evident for the players with the highest match load, while not in the players with the lowest match load. Such conditioning effect could be explained by fitter players perform more running activity (Krustrup et al., 2003), but also recover faster (Johnston, Gabbett, & Jenkins, 2015).

5.2.2 Match load as a predictor of recovery status

The substantial effects of external load variables on CK, myoglobin, and SP30 that were seen at 72 h post-match provide evidence that match load affects the time to recovery. Thus, players with low match load on average recovered at 72 h, while players with high match load did not. Such a finding has important practical applications as tracking devices can be used in managing recovery strategies or training load. Moreover, our data showed that some external load variables could predict recovery on average, but not in individuals based on the wide prediction interval seen on figure 5.3. The wide prediction intervals are a consequence of large individual differences in the recovery, as indicated by the SD on figure 2 of Paper II and figure 5.2. The individual differences in recovery could be a consequence of differences in individual characteristics, such that the same external load produces a different internal load (Paper I). In that case, the internal load may be a better predictor of the recovery status. We did collect internal load with heart rate monitors (chest straps) and RPE in our study in order to test that hypothesis. However we had technical issues with the heart rate straps connection to the OptimEye S5 IMUs and only recorded data from a few participants. We also chose not to use the sRPE data because the participants were not familiar with sRPE, and there was no time to familiarize them due to the short study period. Moreover, based on the data and on experience from study I (data not covered in Paper I), most participants rate matches to 9 or 10 on the RPE scale, and hence very little variability to expect a predictive value.

Some of the variability in the recovery might also be explained by differences in the individual player's relative match load, i.e., the current match load compared to his typical match load over several matches. Given the large within-player, match-to-match variation in external load seen in football (Al Haddad et al., 2018; Carling et al., 2016), some players had presumably a higher relative external load match within this study, while others had lower relative load. A study designed with multiple matches must be carried out to address if differences in within-player external load could predict the recovery from matches more reliably than between-player external load.

5.3 Muscle damage after football match

In Paper III, muscle HSP stress responses, blood markers for muscle damage, muscle soreness, and neuromuscular fatigue were assessed in semi-professional football players 1, 24, 48 and 72 h post-match. The main findings were that (1)

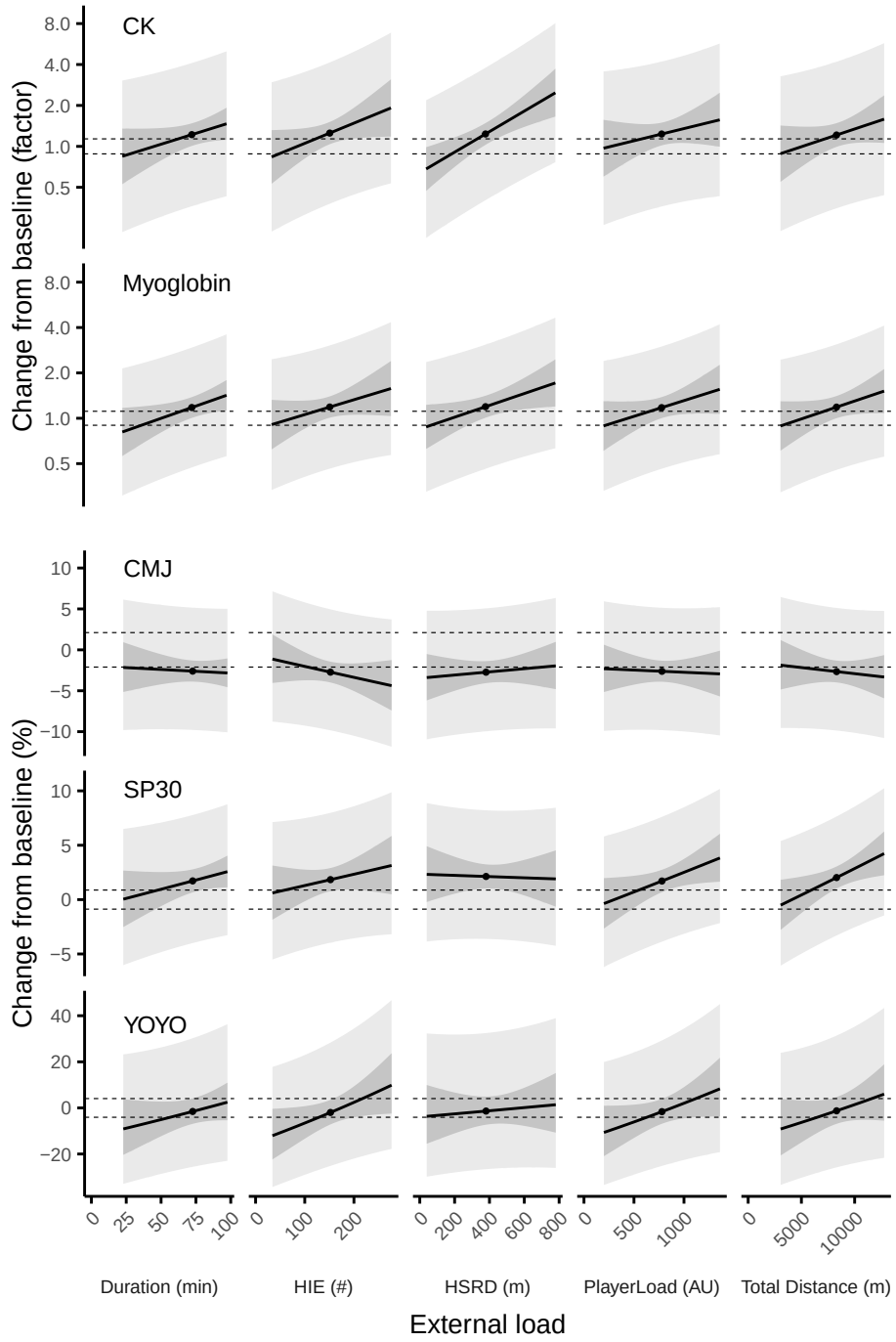


Figure 5.3: Predicted mean change in recovery markers at 72 h post-match for different amounts of match load, after adjustments for baseline and YOYOmax. Match load values are within the range of match load values in this study and mean match load is indicated by a dot symbol. The area between the dotted lines indicates trivial changes; the dark shaded area shows 90 % confidence interval, and the light shaded area shows 90 % prediction interval.

HSP70 and αB-crystallin showed an immediate accumulation in cytoskeletal structures of muscle fibers indicated by an 1.8 and 3.6-fold increase in HSP70 and αB-crystallin in the cytoskeletal fraction at 1 h; (2) a 12–27 % increase in HSP70 and αB-crystallin staining intensity at 24 and 72 h; and (3) the cellular response was accompanied by a decrease in CMJ height, an increase in muscle soreness, and increases in CK and myoglobin blood levels.

5.3.1 Indirect Blood Markers

The increases observed in CK and myoglobin post-match indicates muscle damage which could be categorized as mild exercise-induced muscle damage (Paulsen et al., 2012). The response is comparable to other studies with reserve teams (Russell et al., 2016; Thorpe & Sunderland, 2012) and professional players (Silva et al., 2013), despite that the mean match duration, total and high-intensity distance were lower than observed in a typical full match (Bradley et al., 2013). Furthermore, the response was higher than reported by (de Hoyo et al., 2016), who also included substitutes in their analysis. These comparisons suggest a high response of muscle damage indicators in the current study. Following the same patterns as in a recent meta-analysis (Silva et al., 2018), CK and myoglobin peaked at 24 and 1 h post-match, respectively, and an increase from baseline was still evident after 72 h for CK. Large variations were observed at 72 h, meaning that the muscle damage indicators had returned to baseline in some players, but not in others. For example, two players still had increasing CK at 72 h to over 3200 U/L, suggesting a more severe muscle damage (Paulsen et al., 2012). This is the first study modeling the effect of different external load variables on recovery markers, for a full 72 h time period post-match in football players. The effects, understood as the difference between a typical high and low match load, provide evidence that match load explains changes in the two indicators of muscle damage (figure 4.5). Of the five external load variables, HSRD was the strongest predictor, consistent throughout all time-points. The larger effect of HSRD is supported by other studies where change in CK correlated with high-intensity distance and number of sprints, but not for total distance (de Hoyo et al., 2016; Russell et al., 2016; Thorpe & Sunderland, 2012). The reason for the larger effect could be the high-force and high-speed muscle contractions occurring when maintaining or decelerating from high running speeds, causing muscles to work while lengthening. Such eccentric muscle contractions are shown to cause tearing and disruption of muscle fibers (Paulsen et al., 2012). HIE and PlayerLoad^{TM}, that are based on accelerometer data, could hypothetically assess football-specific movements such as accelerations, decelerations, and change of directions to a higher degree than for example distance covered. Instead, our data show that HIE had a lower effect than HSRD on CK and myoglobin, suggesting that running speed is an important factor for muscle damage. PlayerLoad^{TM} on the other hand had the lowest effects which makes it a poor predictor of muscle damage indicators.

5.3.2 Heat Shock Proteins

The current findings suggest that a 90 min football match elicits a heat shock protein response in the muscle fibers immediately post-match and that it remains elevated 48–72 h after the match. Specifically, an increase in HSP70 and αB-crystallin were observed consistently in both the cytoskeletal fraction of the homogenate and on the muscle cross-sections, which suggests that the HSPs bind to damaged structural proteins (Paulsen et al., 2009). A further indication of damaged structural proteins was the observation of granular staining of αB-crystallin. Such accumulation of HSPs is shown to be co-localized with myofibrillar disruptions (Paulsen et al., 2009). Our results show that approximately 1% of the fibers had granular staining of αB-crystallin at baseline, which doubled to 2.2 % (range: 0–10 % at 1 h post-match. The magnitude of the αB-crystallin response in the cytoskeletal fraction was similar to Frankenberg et al. (2014) where a 30 min eccentric step exercise was performed. However, such translocation of HSPs did not occur following a repeated bout 8 weeks later, suggesting that the HSP response was due to unaccustomed eccentric exercise and that the muscle fibers were protected against muscle damage at the second bout. Such protection is also seen in trained subjects exposed to high-intensity running (Morton et al., 2008). However, in our study, the HSP response was still evident despite that the football players were well trained and were used to playing weekly matches and therefore should be accustomed to the match load. This suggests that the physical load in football matches is substantial and that the intensity in parts of a match exceeds the stress tolerability in some muscle fibers. One explanation for the lack of protection from previous football matches could be that a much broader range of supra-threshold eccentric and concentric movements are executed in football compared to the more uniform movements that are seen in step exercises and running. Such movements would affect a larger range of substructures that may differ from situation to situation and match to match and may not allow for a near full protection. On the other hand, we observed a substantially smaller stress response and less reduction in muscle function after the football matches than has been observed after more extreme muscle-damaging exercise protocols (Paulsen et al., 2007; Vissing et al., 2009). Such protocols have caused HSP27 to increase 10–15 fold in the cytoskeletal fraction and the force-generating capacity in the quadriceps to be substantially decreased even one-week post-exercise.

In both Western blot (cytoskeletal fraction) and immunohistochemistry analyses, HSP70 showed a weaker but longer lasting response than αB-crystallin. In line with other studies (Paulsen et al., 2009), αB-crystallin recovered faster than HSP70 in the cytoskeletal fraction, although this was not evident on the immunohistochemical analyses. The different time patterns may reflect their different roles, as αB-crystallin is thought to be related to acute binding to damaged structural proteins to prevent protein aggregation (Sun & MacRae, 2005), whereas HSP70 is more related to refolding and controlled removal of damaged proteins (Höhfeld et al., 2001). It is well known that HSP70 also binds to newly synthesized proteins helping them to fold to the native state (Mayer

& Bukau, 2005). Hence, we cannot exclude that HSP70 is up-regulated due to increased protein synthesis. The observed external match load and the CK, myoglobin, and CMJ response post-match was comparable to other competitive matches (Bradley et al., 2013; Jones et al., 2019; Rowell et al., 2017; Silva et al., 2018). Hence, it is reasonable to consider the HSP response as representative to a typical football match. The magnitude of the changes observed for CK, myoglobin, muscle soreness, CMJ height, and the HSPs suggests that a typical football match, for the average player, induce muscle damage which by Paulsen et al. (2012) could be categorized as mild. Despite our characterization of muscle damage apparently as mild, the observed recovery time for functional measures, such as CMJ, linear sprint performance, as well as perceptual measures such as muscle soreness, was still in the range of 72 h or more in line with Silva et al. (2018). The concurrent HSPs response along with a reduction in CMJ performance indicates that muscle damage is one of the causes for the prolonged neuromuscular fatigue. It can also be speculated that the mild muscle damage could be the cause of the higher muscle injury rates observed when there is ≤ 3 compared to ≥ 6 days between matches (Bengtsson et al., 2018). In such cases, this mild form of muscle damage is pivotal and must be contended with in practice. Especially those players with the highest match loads and higher risk for more pronounced muscle damage and delayed recovery, should be identified and prioritized in the recovery period between matches.

The rapid decrease of HSPs in the cytosolic fraction and the concurrent increase of HSPs in the cytoskeletal fraction suggest a translocation of the HSPs within the stressed myofibers. It indicates that the HSP changes from an unbound state in the cytosol, to bind to cytoskeletal structures (Koh & Escobedo, 2004). Such translocation is usually seen in other studies after eccentric exercise (Frankenberg et al., 2014; Koh & Escobedo, 2004; Vissing et al., 2009) and muscle-damaging protocols (Paulsen et al., 2009; Paulsen et al., 2007), but not after isometric contractions (Koh & Escobedo, 2004; Vissing et al., 2009). After muscle-damaging protocols we have shown that αB-crystallin accumulates in the Z-disc region, especially in sarcomeres with structural disruptions, and that granular αB-crystallin staining coincides with the sarcomere disruptions (Paulsen et al., 2009). Hence, the observed increase in fibers with granular staining further supports the suggestion that myofibrillar disruptions occurred during matches and contributed to the long-lasting fatigue in this study.

5.3.3 Muscle fiber type-specific stress response

Both the αB-crystallin and the HSP70 responses to football match play seem to be very similar in type I and type II muscle fiber types, also shown by their strong correlations at multiple time points (r =0.88–0.99). This suggests that football match play stresses both muscle fiber types, a pattern that is also seen with glycogen depletion (Krustrup et al., 2022). It could reflect the combination of high-intensity work which activates type II fibers, and the long duration of the match. Stressing both fiber types is in contrast to what is usually observed after high-load resistance exercise (Folkesson et al., 2008), where the stress response

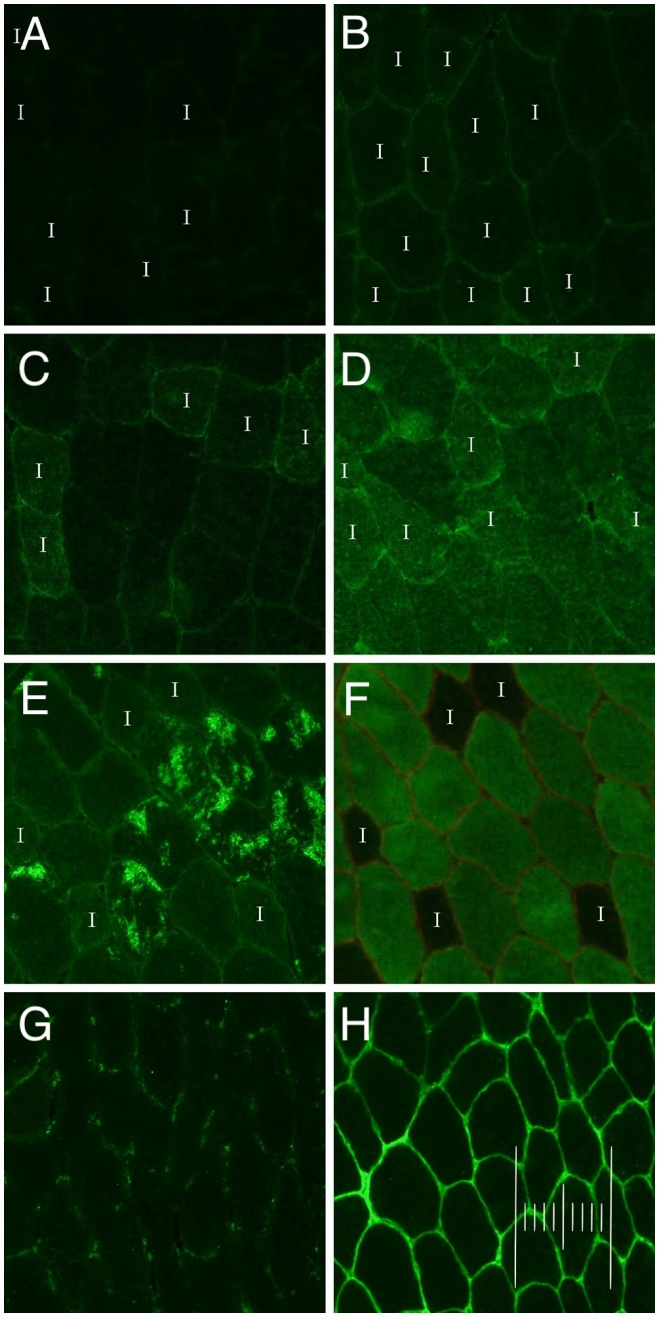


Figure 5.4: Cross-sections of muscle fibers after immunohistochemistry showing: HSP70 staining at baseline (A) and 24 h (B), αB-crystallin staining at baseline (C) and 24 h (D), muscle fibers with substantial granular staining of αB-crystallin at 1h (E) and a corresponding, adjacent cross-section differentiating muscle fiber type I (black) and fiber type II (F), and tenascin-C immunoreactive staining in a subject with substantial differences between baseline (G) and 72 h post-match (H). Fiber type I is labeled on pictures A–F, and a scale bar of 100 µm is printed on picture H

occurred predominantly in type II fibers. On the other hand, low-load, concentric blood-flow restricted exercise, is shown to mainly stress the type I fibers (HSP70) (Cumming et al., 2014). In more extreme muscle-damaging protocols with maximal eccentric muscle action, αB-crystallin and HSP27 responses are seen in both fiber types, while a HSP 70 response was mainly seen in type II fibers (Paulsen et al., 2009). Consequently, the combination of prolonged metabolic stress and repeated short bursts of high-intensity accelerations and decelerations are likely to contribute to the HSP responses observed in both type I and type II fibers after the football match.

5.3.4 Extracellular matrix and inter-individual differences

Exercise may also induce damage to passive, extracellular structures in the muscles. Tenascin-c is a protein that contributes to the remodeling of collagen fibers in the extracellular matrix, and increases in tenascin-c staining have been observed in muscle-damaging exercise protocols along with exercise-induced, intra-cellular damage (Raastad et al., 2010). While an average increase in the immunoreactive area for tenascin-c was observed post-match in the current study, the inter-individual variability was large, which is illustrated by the wide confidence intervals (figure 4.10). In studies showing a clear positive tenascin-c response, the subjects had been subjected to unaccustomed, high-force exercise (Crameri, Langberg, Teisner, et al., 2004; Raastad et al., 2010). The lack of a uniform tenascin-c response in this study could therefore indicate that football matches have a tolerably load for the extracellular matrix that most players are adapted to, but with individual deviations. Individual differences in the response to the match were evident in several of the other measurements. For example, two of the players experienced a very high CK and myoglobin response after the match, combined with a secondary, additional increase at 72 h with CK values of 2400 and 3200 U/L and myoglobin values of 216 and 299 $\mu g\,L^{-1}$. Furthermore, one subject showed extensive granular staining at 1 h post-match (10 % of muscle fibers). These individual cases suggest that some players experienced a more severe exercise-induced muscle damage. Hence, practitioners should have an individual focus, as some players, on some occasions, may have more extensive muscle damage which could require longer recovery time.

5.3.5 Correlations between measurements

The match load in the current study caused significant CK, myoglobin, and CMJ responses comparable to typical football matches (Silva et al., 2018). Furthermore, the HSP stress responses indicated that some ultrastructural muscle damage occurred. While in theory these measures may be linked, the responses in neither the blood markers nor the CMJ were correlated with the response of any of the reported HSP measures. One reason could be that CK and myoglobin also originate from muscles other than m. vastus lateralis. In fact, increase in CK and myoglobin levels post-match was positively related to the amount of high-speed running during the match (Paper II, an activity pattern where the hamstring

musculature is highly activated. In contrast to our study, Paulsen et al. (2007) found a strong correlation between decreased force-generating capacity and the responses of HSP27 (which has similar a response to αB-crystallin) and HSP70 at 0.5 h after exercise. However, in that study the reduction in force-generating capacity ranged from $-20\,\%$ to nearly $-80\,\%$, whereas in our study the range of CMJ response was only -4 to $-15\,\%$ at 1 h post-match, meaning that the signal-to-noise ratio in our study was too low to detect an association. It is also worth pointing out that CMJ includes many muscles other than m. vastus lateralis and are generally a more complex movement, compared to knee-extension that was used in Paulsen et al. (2007). Lastly, it should be acknowledged that the sample size was rather small for correlations analyses, thus, the results should be interpreted with caution.

5.4 Limitations of the studies

Some limitations should be considered when generalizing the results from these studies. In Paper I, the lack of a gold standard to measure training load in team sports makes the criterion validity of both external load variables and sRPE-TL challenging to assess. Consequently, it is possible that we have been comparing two suboptimal measures of training load. Furthermore, despite the reasonable number of players and sessions analyzed, the current study only considered one team. Caution should be made as there could be very different training cultures between teams. Finally, in the calculation of sRPE-TL, sRPE was based on the session as a whole, while the external load and session duration came from the tracking system recordings that excluded any individual warm-up or other activity before or after the session. Hence, such extra activity could possibly influence the sRPE score, but not the external load measures and session duration. However, we doubt that such low-intensity activity had a substantial influence on the sRPE score.

A limitation in Paper II is the single match per player design, which only allowed for between-player modeling of the external load variables. It would have been ideal to have the time and resources to do a multiple-match design that models the effect of *within-player* match load. It could possibly have decreased the uncertainty in the estimates of recovery, and especially the effects of external load variables. In addition, we suspect that the study, especially on the last two time-points, was somewhat underpowered as some of the measures were inherently unreliable. Although the external load and recovery data were regarded as representative, the matches were nonofficial matches, played 2–3 weeks after the season, in a period without other matches and with a lower self-reported training load. Hence, the match load and the recovery from the match might have been different from an official, within-season match. Lastly, the control of the players' physical activity, nutrition strategies, and sleep before and after the match were limited to pre-study instructions from the research staff.

A limitation to the methodology in Paper III is that the muscle tissue from

a needle biopsy is a very small sample compared to the whole muscle, to the muscle group, and to the total number of muscle groups involved in running and jumping. Thus, indications of muscle damage based on the muscle samples could be under- or overrepresented, and inferences are limited to the quadriceps muscle group only. Furthermore, the wide confidence intervals for the biopsy measure estimates could indicate somewhat low power, especially at the 48 h time point with only 7 subjects. The large uncertainty could be partly due to trying to infer from a small muscle sample to a large muscle group, as discussed above and be partly due to sampling and measurement error from the multi-step laboratory analyses. Also, real differences in responses between players due to individual characteristics (i.e., training status, age, and genetics), different playing positions, or differences in the external match load relative to their typical external match load could have contributed to the uncertainty. Due to the invasiveness of the study design, the matches were conducted two to three weeks after the end of the season, meaning that the training load before the match might have been different from a typical in-season match. Nevertheless, the players were still training in this period, but they completed fewer sessions per week and no matches.

Chapter 6

Practical Applications

- We recommend sRPE-TL as a valid tool for quantifying training load based on its ability to discriminate between different amounts of external load.
- Practitioners should use external load variables with no or low intensity-thresholds, such as total distance, PlayerLoad^{TM}, PlayerLoad2D^{TM}, and HIE > 1.5, when single variables are used to describe training load.
- External load variables with high intensity-thresholds are unsuitable alone to describe training load because of weaker relationships with sRPE-TL and its poor ability to explain the between-session variability in sRPE-TL.
- Individual responses to external load highlight the importance of having an individual focus when analyzing and managing training load. Furthermore, the individual responses, between-player differences and the remaining between-session variability suggest that both sRPE-TL and external load should be monitored.
- In matches, HSRD is associated with subsequent muscle damage and the amount of HSRD could be used as a determining factor when managing training load and recovery strategies between matches.
- The mild muscle damage we observed from football matches, lasting 48–72 h, suggests that match schedules should have a minimum of 72 h between matches.

Chapter 7

Conclusions

From the studies the following conclusions are drawn. There is a close relationship between external load variables extracted from player tracking systems, and internal load assessed by the sRPE-TL method. External load variables with no intensity-threshold or low intensity-threshold had the strongest relationships with sRPE-TL. Furthermore, large individual response in sRPE-TL to external load variables was observed, which highlights the importance of individualized monitoring of training load and advocates for the use of both external load and internal load in training load monitoring. However, even for the external load variables with the strongest relationships with sRPE-TL, there was large between-session variability in sRPE-TL that could not be explained by external load variables.

External load variables derived from player tracking systems have an effect on recovery markers up to 72 h post-matches. HSRD had the most substantial effect on muscle damage indicators, and PlayerLoad^{TM} and total distance affected sprint performance. Hence, a combination of several different tracking device variables is advised to ensure a better representation of the match load. Unexpectedly, the external load variables showed no dose-response relationships with neuromuscular fatigue, measured by CMJ, despite CMJ performance where significantly lower post-match. While the mean changes in recovery markers approached baseline values at 72 h post-match, the effects of external load variables on the same recovery markers were still substantial, suggesting that external load variables could partly explain the time to recovery. Despite these substantial mean effects, it was not possible to predict the recovery of individual players at 72 h from any of the external load variables due to too much uncertainty in the predictions.

Football match play produced a muscular HSP stress response, increases in markers of muscle damage in blood, reduced CMJ performance, and increased perceived muscle soreness compatible with mild muscle damage. Such muscle damage could contribute to the prolonged recovery time after football matches. Specifically, the observation of HSPs accumulation in cytoskeletal structures and increased proportion of fibers with granular HSP staining indicates damage to myofibrillar proteins. Furthermore, football match play seems to stress both type I and type II muscle fiber types similarly. However, compared to experiments with muscle-damaging protocols or with protocols where the task is unaccustomed, the HSP stress response was moderate. Consequently, the players are adapted to football match play, but there are still loading patterns in match play that exceeds the tolerability threshold and results in muscle damage. Both the variation in individual match load and training status probably contribute to the large variation in neuromuscular fatigue and time needed for full recovery.

Overall, the data suggests a relationship between external load and internal

load, and external load and muscle damage and recovery. These studies highlight individual differences in the response to external load, and the importance of an individual approach to player load monitoring, and to utilize several variables, representing both internal and external load to obtain a more complete picture of the "true" load. Based on the relationships between load and recovery, player monitoring may assist to improve recovery strategies, especially in periods with frequent matches.

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Papers

Paper I

Individual Response to External Training Load in Elite Football Players

I

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Human Kinetics
ORIGINAL INVESTIGATION

Individual Response to External Training Load in Elite Football Players

Håvard Wiig, Thor Einar Andersen, Live S. Luteberget, and Matt Spencer

Purpose: To investigate within-player effect, between-player effect, and individual response of external training load from player tracking devices on session rating of perceived exertion training load (sRPE-TL) in elite football players. ***Methods:*** The authors collected sRPE-TL from 18 outfield players in 21 training sessions. Total distance, high-speed running distance (>14.4 m/s), very high-speed running distance (>19.8 m/s), PlayerLoad™, PlayerLoad2D™, and high-intensity events (HIE > 1.5, HIE > 2.5, and HIE > 3.5 m/s) were extracted from the tracking devices. The authors modeled within-player and between-player effects of single external load variables on sRPE-TL, and multiple levels of variability, using a linear mixed model. The effect of 2 SDs of external load on sRPE-TL was evaluated with magnitude-based inferences. ***Results:*** Total distance, PlayerLoad™, PlayerLoad2D™, and HIE > 1.5 had most likely substantial within-player effects on sRPE-TL (100%–106%, very large effect sizes). Moreover, the authors observed likely substantial between-player effects (12%–19%, small to moderate effect sizes) from the majority of the external load variables and likely to very likely substantial individual responses of PlayerLoad™, high-speed running distance, very high-speed running distance, and HIE > 1.5 (19%–30% coefficient of variation, moderate to large effect sizes). Finally, sRPE-TL showed large to very large between-session variability with all external load variables. ***Conclusions:*** External load variables with low intensity-thresholds had the strongest relationship with sRPE-TL. Furthermore, the between-player effect of external load and the individual response to external load advocate for monitoring sRPE-TL in addition to external load. Finally, the large between-session variability in sRPE-TL demonstrates that substantial amounts of sRPE-TL in training sessions are not explained by single external load variables.

Keywords: internal load, individual differences, variability, team sport

Monitoring and managing training load may assist to achieve the desired training outcome[1] and reduce injury risk.[2,3] However, quantifying training load accurately and reliably is challenging in team sports due to the complexity of movements and actions, and the constant shifting intensities. Training load is typically classified into external load, defined as the work completed by an athlete measured independently of his or her internal characteristics, or into internal load, defined as the relative physiological stress imposed on the athlete.[4] Hence, the internal load is determined by an interaction of the external load and the individual characteristics of the athlete.[5] Internal load is commonly represented by heart rate–derived training impulse, session rating of perceived exertion (sRPE), or sRPE training load (sRPE-TL), where sRPE-TL seems to have the strongest relationship with external load.[6]

The sRPE-TL method is an easy, low-cost method of measuring internal load that has been validated in football.[7,8] Its reliability, on the other hand, is questionable. Reliability measurements from running[9] and cycling activity[10] have shown poor outcomes, and reliability measurements from football field sessions have not yet been reported. Over the past decade, development and adoption of player-tracking devices with global navigation satellite systems and inertial measurement units have made it easy to quantify external load with acceptable validity[11,12] and reliability.[13,14] However, external load does not consider the individual characteristics, such as genetic factors and training experience. Furthermore, the information extracted from these tracking device systems are divided somewhat arbitrarily into external load variables, such as total distance, high-speed running distance (HSRD), accelerometer-based load, accelerations, and decelerations, and where any single external load variable only covers parts of the overall external load. Thus, how these external load variables affect the internal load in football is not fully understood.

In a meta-analysis comparing single external load variables to sRPE-TL in team sports, total distance covered ($r = .79$; 90% confidence interval [CI], .74 to .83) and PlayerLoad™ ($r = .63$; 90% CI, .54 to .70) show the highest correlations, whereas HSRD ($r = .47$; 90% CI, .32 to .59) and very high-speed running distance (VHSRD, $r = .25$; 90% CI, .03 to .45) show lower correlations.[6] Attempts have previously been made to combine several external load variables to predict sRPE-TL in multiple regression analyses[15]; however, they explained no more variance than, for example, total distance or PlayerLoad™ do alone.[16] More research is needed to clarify these relationships. Furthermore, the magnitude of the correlation coefficient seems to vary with training mode. McLaren et al[6] found that skills and neuromuscular training had possibly moderate to large reductions in the correlation coefficient compared with mixed training mode, while the difference between mixed and metabolic training was unclear. Due to differences in individual characteristics between players, several studies have chosen to analyze within-subject relationships between internal load and external load.[15,17] Nevertheless, little focus has been placed on how players differ in the relationship between external load and internal load. For example, individual players could vary in which external load variable was the most important descriptor of sRPE[18] implying that players have different internal load

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responses to the same external load variables. The magnitude of the individual response to external load has, however, not been previously investigated.

In this study we aimed, first, to model the within-player and the between-player effects of different external load variables on sRPE-TL in elite football. Second, to model the magnitude of individual differences in the response to external load, and finally, to investigate the variability in sRPE-TL that is not explained by external load.

Methods

Subjects

A total of 18 male players from one football team (age 26 [5] y, height 183 [6] cm, body mass 80 [9] kg), participating in the Norwegian Premier league, took part in this study. The group of players included 7 defenders, 5 midfielders, and 6 attackers. All players provided written informed consent according to the Declaration of Helsinki. The study was approved by the Norwegian Centre for Research Data.

Design

We designed the study to compare measures of sRPE-TL and external load for a team of players during the in-season competition period, from March to November (32 wk). A total of 207 individual training observations from 21 training sessions were included, with a median of 10 (4) observations per player (range 7–18). The training sessions were all on-field sessions, with a duration of 75 (11) minutes, excluding any individual preparations, warm-ups, or drills before and after the session. All sessions were performed on the same football pitch covered with third generation artificial turf.

Methodology

Collection of sRPE-TL. Each player reported their sRPE via a mobile app (PMSYS; University of Oslo, Oslo, Norway) 45 minutes (median) after the session, using a modified Borg CR-10 scale[7] with integers and verbal anchors. sRPE-TL was calculated by multiplying the sRPE by the session duration in minutes, defined by the start and stop from the tracking system recordings. We instructed the players to consider each training session as multiple small periods with a hypothetical rating of perceived exertion (RPE) of each period. The sRPE should represent an average of all the hypothetical RPEs throughout each session.

Tracking of External Training Load. Each player was equipped with a tracking device (OptimEye S5, Firmware 7.18; Catapult Sports, Melbourne, Australia), located between the scapulae in a manufacturer-provided vest. The device was specified with a nondifferential, 10-Hz global navigation satellite system and a 3-dimensional accelerometer, magnetometer, and gyroscope, all operating at 100 Hz. There was some interchange of devices between players, resulting in that players used the same device for approximately 50% of the sessions. All the devices were calibrated according to the manufacturer's instructions prior to the commencement of the study. The devices were turned on outdoors, 15 minutes before data collection commenced.

Data Processing. We extracted the raw data from the tracking devices after each session using the Catapult Sprint software (version 5.1.7; Catapult Sports). Eight different tracking device variables were chosen to provide different representations of the actual external training load. Of these, PlayerLoad™, PlayerLoad2D™, total distance, and HIE > 1.5 were regarded as variables with low intensity-thresholds, whereas HSRD, VHSRD, HIE > 2.5, and HIE > 3.5 were regarded as variables with high intensity-thresholds. PlayerLoad™ is a vector magnitude expressed in arbitrary units as the square root of the sum of the squared instantaneous rate of change in acceleration in 3 dimensions, described more comprehensively by Boyd et al.[19] PlayerLoad2D™ excludes the vertical dimension. High-intensity events (HIE) are the sum of acceleration, deceleration, and change of direction events exceeding a threshold of either 1.5 m/s (HIE > 1.5), 2.5 m/s (HIE > 2.5), or 3.5 m/s (HIE > 3.5), based on procedures by Luteberget and Spencer.[13] During indoor field assessment, PlayerLoad™, PlayerLoad2D™, HIE > 1.5, HIE > 2.5, and HIE > 3.5 have shown a between-device coefficient of variation (CV) of 0.9%, 1.0%, 1.8%, 3.1%, and 5.5%, respectively.[20] Three variables of total distance covered were categorized into total distance (>0 m/s), HSRD (>14.4 m/s), and VHSRD (>19.8 m/s). The between-device reliability of total distance variables with different thresholds have been estimated with CVs of 1.5% (>0 m/s), 0.6% (3–5 m/s), and 1.0% (>5 m/s).[14] One player missed 5 sessions of HIE data, and another player missed 1 session with total distance data due to failure of 2 devices.

Statistical Analysis

The data were analyzed using a linear mixed-effects model (The MIXED procedure in SAS software, version 9.4; SAS Institute, Cary, NC). The sRPE-TL was treated as the response variable and log-transformed to reduce bias due to nonuniformity of errors. All effects were back-transformed to percent effects. The external load variables were treated as predictor variables, and separate analyses was conducted for each predictor variable. The HSRD and VHSRD predictors were log-transformed to address nonlinearity. Two fixed-effect parameters were specified to separate within-player and between-player effects of the external load variable on sRPE-TL. To obtain the within-player effect, the external load variable was centered to the mean of each player. To obtain the between-player effect, the individual player's mean external load of all sessions was repeated for each observation of sRPE-TL. The model was specified with random intercept for playerID and random slope for playerID × predictor (with an "unstructured" covariance structure), as well as random intercept for sessionID. We allowed for negative variances to estimate realistic confidence limits for the variances and the SD derived. The random effects are presented as SD (in percentage) and represent pure between-player variability (playerID), individual response to 2 SDs of the predictor (playerID × predictor), between-session variability (sessionID), and within-player variability in a typical session (residuals).

The predictors were centered and rescaled to a SD of 0.5 to properly evaluate the magnitude of the effect of continuous variables.[21] A 2-SD gauge of the effects can be justified as the difference between a typical high and a typical low load training session (within-player fixed effect), and the difference between players with typical high and a typical low average external load (between-player fixed effect). The magnitudes of the effects are presented as standardized effect sizes (the effects divided by the square root of the sum of the playerID and residual variances), where <0.2, 0.2 to 0.6, 0.6 to 1.2, 1.2 to 2.0, and >2.0 are regarded as trivial, small, moderate, large, and very large effects,

respectively. For interpreting random effects, which are SDs, these thresholds are halved.[21] Nonclinical, magnitude-based inferences were used, where an effect was deemed unclear if the 90% CI included small positive and negative effects; the effect was otherwise deemed clear. Qualitative assessment of chances of clear outcomes was as follows: >25% to 75%, possibly; >75% to 95%, likely; >95% to 99%, most likely.[21]

Results

A summary of the training load is presented in Table 1. The low intensity-threshold variables showed a 100%–106%, very large within-player effect on sRPE-TL (Table 2). Furthermore, large to very large (very likely substantial) individual responses in sRPE-TL were observed to PlayerLoad™, PlayerLoad2D™, HSRD, and VHSRD (Table 3 and Figure 1). Moreover, we observed 18%–20%, moderate (likely to very likely substantial) between-player effects of PlayerLoad™, total distance, HSRD, and VHSRD on sRPE-TL (Table 2 and Figure 2). After adjusting for the external load variables, 21%–29%, large (most likely substantial) between-session variability was observed in the models with the low intensity-threshold variables, whereas 35%–54%, very large (most likely substantial) between-session variability was observed in the models with the high intensity–threshold variables. Finally, sRPE-TL showed a within-player CV of 23% (90% CI, 21 to 26) in a typical session, when adjusted for either PlayerLoad™, PlayerLoad2D™, or total distance (Table 3).

Discussion

In this study, we modeled the effect of external load variables on sRPE-TL during training sessions in elite football players, using an individual approach. We found that external load variables with low intensity-thresholds were closely related to sRPE-TL; however, the relationship became weaker with increasing intensity thresholds. Furthermore, small to moderate between-player effects of external load were evident for most of the external load variables. Finally, the data show moderate to large individual responses to PlayerLoad™, PlayerLoad2D™, HIE > 1.5, HSRD, and VHSRD. Although external load had large to very large within-player effects on sRPE-TL, there was still large to very large between-session variability in sRPE-TL, as well as between-player variability and within-player variability that could not be explained by external load variables.

Our results show that sRPE-TL could differentiate 2 SDs of the external load variable, corresponding to the difference between a typical low- and high-load session (large to very large effect size). In fact, even 0.5 SD change in PlayerLoad™ and total distance led to an approximate 20% difference in sRPE-TL (moderate, very likely to most likely substantial effects) from these variables (data not shown). The ability of sRPE-TL to discriminate between different amounts of external load within the same player suggests that sRPE-TL is a valid tool quantifying internal training load, in accordance with existing literature.[6–8]

Session rating of perceived exertion training load had the strongest relationship with the external load variables with no threshold or low intensity-thresholds, that is, PlayerLoad™, PlayerLoad2D™, total distance, and HIE > 1.5 (Table 2), in agreement with other studies on team sports.[6,9,15,16,22,23] This finding suggests that the sRPE-TL first and foremost reflects the total work completed, rather than periods of high-intensity work. The reason could be that both sRPE-TL and the low intensity-threshold variables are strongly dependent on the session duration because all work is quantified regardless of intensity, whereas the high intensity–threshold variables are more related to the duration of high-intensity work. The external load variables with high intensity-thresholds (HSRD, VHSRD, HIE > 2.5, and HIE > 3.5) showed weaker relationships with sRPE-TL, although still large, most likely substantial effects were evident. While part of the weaker relationship could reflect the decreased accuracy of the global navigation satellite systems with increased speed,[24] it is more likely due to the small fraction of the total work covered at high intensity in the training session. In fact, many sessions had very little high-intensity work at all (Table 1). In addition, in some players these variables had a negative effect on sRPE-TL (Figure 1). Interestingly, compared with a reference model without any external load predictors, these variables explained very little of the between-session variability and also less of the within-player variability in sRPE-TL than the low intensity-threshold variables (Table 3). From these perspectives, variables describing high-intensity work is not only inferior, but also unsuitable as single predictors of training load, particularly when training regimes include low-intensity training sessions. This does not mean that high-intensity work does not contribute to training load. High-intensity work is self-evidently demanding, and high-intensity external load variables have successfully been used in multiple regression to predict sRPE-TL[17] and in machine learning models predicting pure RPE.[18,25] More studies are indeed needed, especially on predicting sRPE-TL using machine learning techniques.

The between-player effect describes the average difference in sRPE-TL between players with a typically low and typically high mean external load. The variables based on PlayerLoad™ and distance showed borderline small to moderate, likely substantial effects, indicating that players who do more external training load on average also report higher sRPE-TL. Hence, the individual players' average external load does not result in having the same sRPE-TL. Therefore, using external load alone is probably not sufficient when monitoring individual training load, as it overlooks the differences in internal load between players. It could be that players with high average external load are pushing themselves harder and, therefore, report a higher sRPE-TL than players with low average external load. In that case, the sRPE-TL works as intended. The between-player effect could possibly be biased by that some players participated more in the sessions with high training load and others more in the sessions with low training loads. However, this possible bias is accounted for in the statistical model by the random intercept for session ID. Although small to moderate between-player effects of external load were found, there was still 13%–20% between-player variability in sRPE-TL that could not be explained by the external load variables (Table 3), reflected by the wide CIs in Figure 2. No substantial between-player effects were found for HIE > 2.5 and HIE > 3.5.

An important finding in this study was the individual response in sRPE-TL to external load, represented as individual slopes in Figure 1 and with individual data in Figure 3. Two SDs of PlayerLoad™, PlayerLoad2D™, HSRD, and VHSRD resulted in large (very likely substantial) variability in sRPE-TL response, whereas HIE > 1.5 resulted in a moderate (likely substantial) variability in sRPE-TL response (Table 3). In practice, this means that, for example, a 224 arbitrary unit increase in PlayerLoad™ will lead to 106.4% (24%) increase in sRPE-TL for a group of players (Tables 2 and 3). This finding supports the theory that

Table 1 Summary Statistics of the Training Load Variables Grouped by All Observations, Mean of All Players, and Mean of All of Sessions

Group	sRPE, AU	Duration, min	sRPE-TL, AU	PlayerLoad™, AU	PlayerLoad2D™, AU	Total distance, m	HSRD, m	VHSRD, m	HIE > 1.5, n	HIE > 2.5, n	HIE > 3.5, n
All observations											
Mean	4.1	75.2	322	467	274	4293	388	108	616	164	47
SD	1.6	13.1	159	125	71	1089	246	133	190	59	21
Min	1.0	38.0	45	174	104	1580	3	1	179	32	5
Max	9.0	98.0	855	872	518	7345	1253	779	1441	425	137
Mean of players											
Mean	4.1	75.2	320	469	274	4284	393	108	621	165	47
SD	0.7	2.5	62	61	31	410	108	55	84	33	13
Min	2.6	70.0	191	334	201	3752	244	54	520	123	28
Max	5.4	78.6	442	563	318	5184	578	210	836	241	78
Mean of sessions											
Mean	4.2	75.4	325	469	275	4305	391	111	618	163	46
SD	1.2	11.4	126	99	57	903	181	101	152	43	14
Min	2.0	45.2	96	217	134	1813	162	21	306	72	17
Max	6.8	93.7	618	652	383	5720	853	439	898	258	81

Abbreviations: AU, arbitrary unit; HIE, high-intensity events; HSRD, high-speed running distance; min, minimum; max, maximum; sRPE, session rating of perceived exertion; sRPE-TL, sRPE training load; VHSRD, very high-speed running distance.

Table 2 The Within-Player and Between-Player Effect of the Specific External Load Variable on sRPE-TL

External load variable	Value of 2 SDs	Effect, %	90% CI	ES
Within-player effect				
PlayerLoad™, AU	224	106.4	83.3 to 132.5	2.60***
PlayerLoad2D™, AU	130	102.0	79.8 to 127.1	2.52***
Total distance, m	2011	100.6	82.5 to 120.5	2.68***
ln(HSRD)[a]	1.48	47.4	30.3 to 66.6	1.40***
ln(VHSRD)[a]	2.58	39.5	20.7 to 61.2	1.18***
HIE > 1.5, n	346	100.3	77.6 to 125.7	2.37***
HIE > 2.5, n	100	75.0	55.7 to 96.7	1.92***
HIE > 3.5, n	33.7	52.4	37.4 to 68.9	1.39***
Between-player effect				
PlayerLoad™, AU	122	19.4	3.2 to 38.1	0.64*
PlayerLoad2D™, AU	62	16.1	0.6 to 34.0	0.54*
Total distance, m	820	17.5	2.7 to 34.5	0.62*
ln(HSRD)[a]	0.72	19.8	5.9 to 35.7	0.65**
ln(VHSRD)[a]	1.39	19.3	5.5 to 34.9	0.63*
HIE > 1.5, n	168	12.4	−2.1 to 29.1	0.40*
HIE > 2.5, n	66	3.9	−9.2 to 19.0	0.13
HIE > 3.5, n	27	0.1	−13.2 to 15.5	0.00

Abbreviations: AU, arbitrary unit; CI, confidence interval; ES, effect size; HIE, high-intensity events; HSRD, high-speed running distance; sRPE-TL, sRPE training load; VHSRD, very high-speed running distance. Note: Uncertainty is indicated by 90% CI. The effect is gauged by 2 SDs of the external load variable.
[a]Natural log transformation. The likelihoods of a clear outcome are: *likely, **very likely, and ***most likely.

Table 3 Random Effects Describing the Variability in sRPE-TL That Is Not Explained by the Specific External Load Variable

	Between-player variability		Within-player variability		Between-session variability			Individual response		
External load variable	CV, %	90% CI	CV, %	90% CI	CV, %	90% CI	ES	CV, %	90% CI	ES
Without predictor	14	5 to 19	35	32 to 40	55	34 to 73				
PlayerLoad™	20	11 to 27	23	21 to 26	21	13 to 27	0.68***	24	10 to 33	0.76**
PlayerLoad2D™	20	11 to 26	23	21 to 26	21	13 to 28	0.69***	23	9 to 32	0.73**
Total distance	16	9 to 22	23	21 to 26	24	14 to 31	0.81***	13	−7 to 21	0.47
HSRD	13	6 to 18	28	25 to 31	49	31 to 65	1.45***	22	5 to 32	0.72**
VHSRD	13	5 to 18	28	26 to 32	54	34 to 72	1.55***	30	5 to 44	0.93**
HIE > 1.5	19	10 to 26	26	23 to 29	29	18 to 38	0.87***	19	3 to 28	0.59*
HIE > 2.5	17	8 to 23	27	25 to 31	35	22 to 46	1.04***	17	−8 to 27	0.55
HIE > 3.5	16	7 to 22	30	27 to 34	40	25 to 53	1.12***	11	−9 to 19	0.34

Abbreviations: CI, confidence interval; CV, coefficient of variation; ES, effect size; HIE, high-intensity events; HSRD, high-speed running distance; sRPE-TL, session rating of perceived exertion training load; VHSRD, very high-speed running distance. Note: Thresholds for ESs for random effects are: >0.1, small; >0.3, moderate; >0.6, large; >1.2, very large; and >2.0, extremely large. The random effects are separated into within-player, between-player, and between-session variability, and individual response to external load, all presented as CV% with 90% CIs. Between-session variability and individual response are evaluated by magnitude-based inferences. The likelihoods of a clear outcome are: *likely, **very likely, and ***most likely.

internal load is determined by external load in interaction with individual characteristics.[5] The individual differences in sRPE-TL response underlines the importance of individualized monitoring of training load in team sports and the need for monitoring internal load in addition to external load. While the current study did not assess individual characteristics of players, other studies have found that individual characteristics such as experience, position, and time-trial performance functioned as mediators of the relationship between external load and sRPE-TL in Australian rules football,[23] and athletes with greater maximal oxygen uptake seem to rate lower sRPE-TL and sRPE.[26,27] These studies as well as the current study highlight the individual differences when rating sRPE-TL. Contradictory to our results, Jaspers et al[25] found that prediction of pure sRPE from external load variables using machine learning techniques could be made more accurate from models on a group of players, than from models on individual

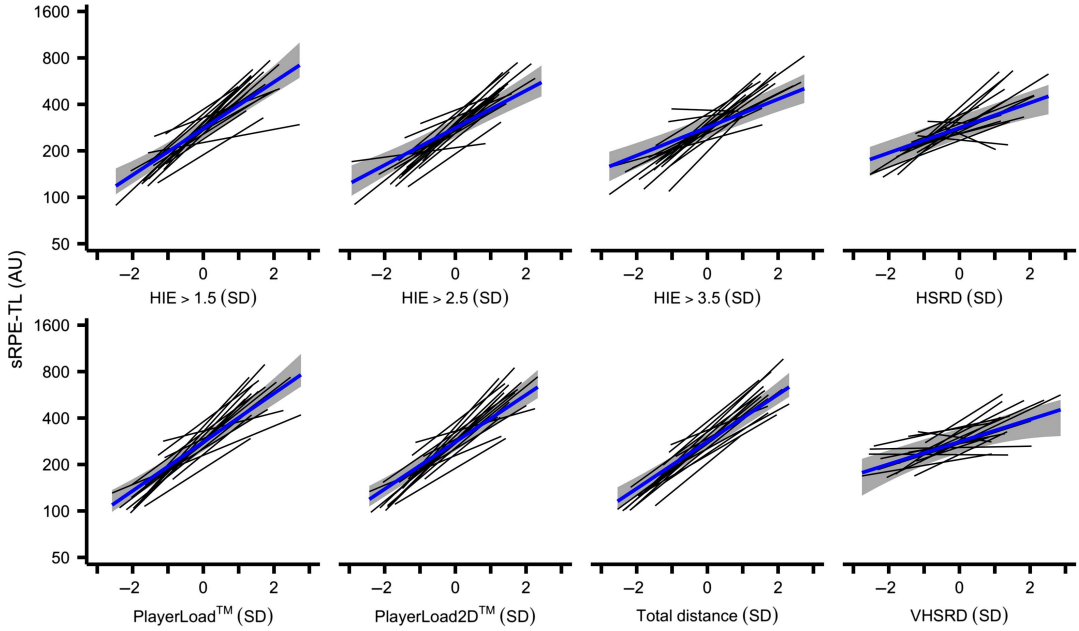

Figure 1 — sRPE-TL predicted by external load highlighting individual response (thin lines), for all external load variables. The solid regression lines are the within-player effect with 90% confidence intervals (shaded area). The *x*-axis shows the external load in number of SDs relative to the individual players' mean external load. The *y*-axis on all panels has a logarithmic scale. HIE indicates high-intensity events; HSRD, high-speed running distance; sRPE-TL, session rating of perceived exertion training load; VHSRD, very high-speed running distance.

players. The reason for the higher accuracy with the group models could be because of far larger sample size compared with the models on individuals. In addition, the external load relationships with sRPE could be different than with sRPE-TL. Interestingly, the individual response observed for total distance was lower and unclear. This makes total distance more uniformly related to sRPE-TL across players. If such findings are consistent, and given that total distance has the strongest correlation with sRPE-TL, total distance seems to be the most preferable training load measure when a single measure is used.

The random effects from the model are estimations of the variability in sRPE-TL that were not explained by the external load variables (Table 3). The between-session variability represents the unexplained variability in sRPE-TL due to that every session is different. It is clear that the between-session variability increases as the intensity threshold for the external load variables increases. The poor ability to explain the between-session variability of sRPE-TL suggests that the high intensity–threshold variables to be unsuitable as single predictors of sRPE-TL when monitoring multiple training sessions. Furthermore, the between-session variability highlights that even the best external load variables fail to cover some of the overall external load completed in the different sessions. This could be due to the arbitrary extraction of the external load variables from the tracking devices, or the lack of sensitivity in the tracking devices to measure the overall external load. Hence, valuable information about external load is probably lost when using single external load variables. The sensitivity of external load variables to measure the overall external load may be dependent on training mode. In fact, in the meta-analysis from McLaren et al,[6] training mode was moderating the relationships between external load variables and sRPE-TL. This is also demonstrated earlier by variability in correlation coefficient between sRPE-TL and external load[15,28] and sRPE-TL and HR[29] across different training drills. The effect of training mode was not evaluated in this study.

Despite its widespread use, the reliability of the sRPE method is scarcely researched. In studies with standardized running and cycling protocols at different intensities, sRPE showed poor reliability with CVs of 28%–32%.[9,10] The challenge of reproducing the same training load in repeated sessions makes it difficult to assess the reliability of sRPE-TL method from field sessions. In this study, the within-player variability represents the individual players' variability in sRPE-TL in a typical session, after adjusting for the external load. Thus, we propose a reasonable estimate of the reliability of sRPE-TL of 23% (90% CI, 21% to 26%). The validity of this estimation is however dependent on the degree that the external load variable and random effect for session ID adjust adequately for the differences in true training load between sessions, and that the individual players' characteristics are consistent across the study period. Reasons for the poor reliability of sRPE-TL are for now speculations, but could include several factors. Some variability could come from the crude 10-point scale of the RPE.[30] Players could also find it hard to compare or rank the intensity between sessions and, therefore, not be consistent in the rating of the RPE score. In addition, different recovery status before trainings, change in fitness status during the season,

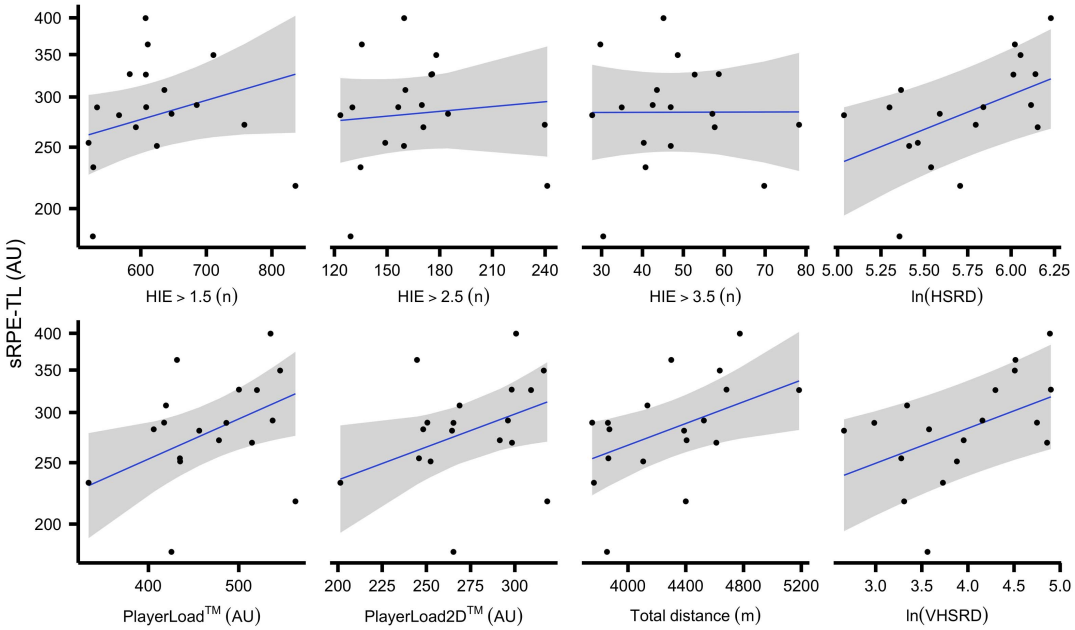

Figure 2 — The individual players' sRPE-TL predicted by their mean external load value, for all external load variables. The regression line is the between-player effect of external load variables on sRPE-TL, with 90% confidence intervals (shaded area). The *y*-axis on all panels has a logarithmic scale. HSRD and VHSRD are transformed to their natural log. HIE indicates high-intensity events; HSRD, high-speed running distance; sRPE-TL, session rating of perceived exertion training load; VHSRD, very high-speed running distance.

or the ability of the external load variables to reliably explain the true training load undertaken by the players could also affect the reliability. It is clear that a comprehensive reliability study is needed to investigate the potential reasons for poor reliability of sRPE-TL.

Some limitations should be considered when generalizing the results from this study. The lack of a gold standard to measure training load in team sports makes the criterion validity of both external load variables and sRPE-TL challenging to assess. As a consequence, it is possible that we have been comparing 2 suboptimal measures of training load. Furthermore, despite the reasonable number of players and sessions analyzed, the current study only considered 1 team. Caution should be made as there could be very different training cultures between teams. Finally, in the calculation of sRPE-TL, sRPE was based on the session as a whole, while the external load and session duration came from the tracking system recordings that excluded any individual warm-up or other activity before or after the session. Hence, such extra activity could possibly influence the sRPE score, but not the external load measures and session duration. However, we doubt that such low-intensity activity had a substantial influence on the sRPE score.

Practical Applications

- We recommend sRPE-TL as a valid tool for quantifying training load based on its ability to discriminate between different amounts of external load.
- Practitioners should use external load variables with no or low intensity-thresholds, such as total distance, PlayerLoad™, PlayerLoad2D™, and HIE > 1.5, when single variables are used to describe training load.
- External load variables with high intensity-thresholds are unsuitable alone to describe training load because of weaker relationships with sRPE-TL and its poor ability to explain the between-session variability in sRPE-TL.
- Individual responses to external load highlight the importance of having an individual focus when analyzing and managing training load. Furthermore, the individual responses and between-player differences suggest that both sRPE-TL and external load should be monitored.

Conclusions

This study adds in the understanding of the relationship between external load and internal load via sRPE-TL in football. The external load variables with no threshold or low intensity-threshold had the strongest relationships with sRPE-TL. Furthermore, large individual response in sRPE-TL to external load variables was observed, which highlights the importance of individualized monitoring of training load and advocates for the use of both external load and internal load in training load monitoring. Finally, even for the external load variables with the strongest relationships with sRPE-TL, there was large between-session variability in sRPE-TL that could not be explained by external load variables.

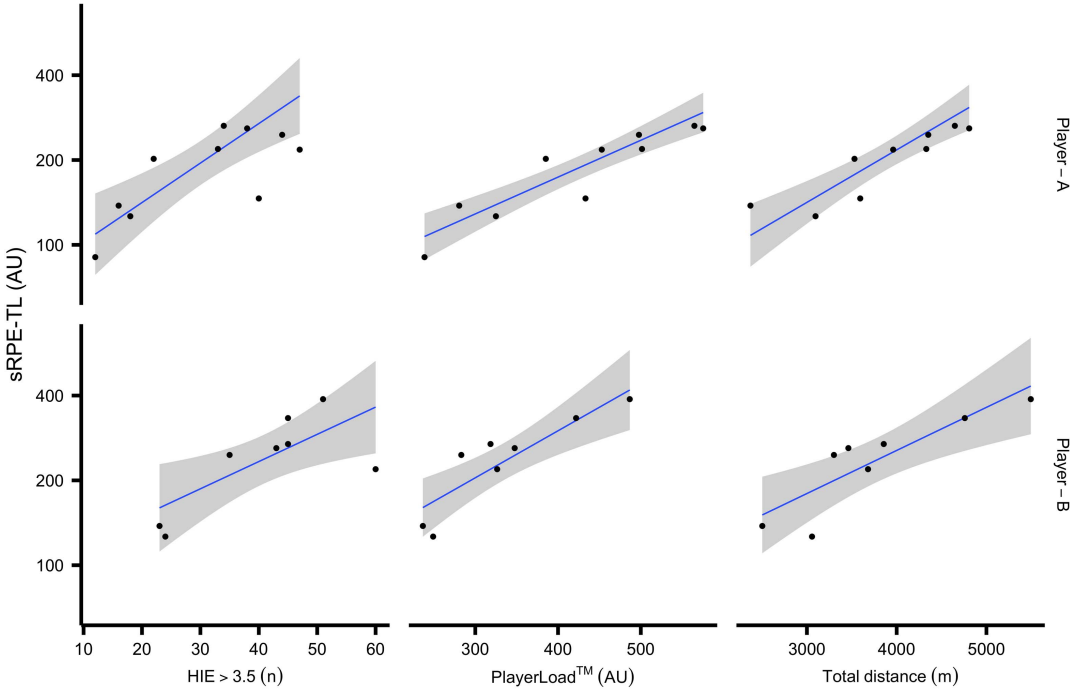

Figure 3 — Session rating of perceived exertion training load (sRPE-TL) predicted by external load for 3 different external load variables, in 2 different players. Uncertainty is indicated by 90% confidence intervals (shaded area). HIE indicates high-intensity events.

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Paper II

External Load Variables Affect Recovery Markers up to 72 h After Semiprofessional Football Matches

Håvard Wiig, Truls Raastad, Live S. Luteberget, Ingvar Ims and Matt Spencer

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External Load Variables Affect Recovery Markers up to 72 h After Semiprofessional Football Matches

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Background: Player tracking devices are commonly used to monitor external load from training and matches in team sports. Yet, how the derived external load variables relate to fatigue and recovery post-training or post-match is scarcely researched. The objective was, therefore, to investigate how external load variables affect recovery markers up to 72 h post-match.

Methods: Semiprofessional players from six teams wore tracking devices during three experimental football matches. External load variables including individual playing duration, total distance, PlayerLoad™, high-intensity running, and high-intensity events were derived from the tracking devices, and blood samples and performance tests from 24–59 players were undertaken post-match. The effect of the external load variables on creatine kinase, myoglobin, and countermovement jump at 1, 24, 48, and 72 h, and 30-m sprint and Yo-Yo intermittent recovery tests level 1 at 72 h post-match, were modeled. Effects were gauged as two standard deviations of the external load and interpreted as the difference between a typical high-load and a typical low-load match. The effects were evaluated with 90% confidence intervals and magnitude-based inferences.

Results: High-intensity running had very likely substantial effects on creatine kinase and myoglobin (moderate factor increases of 1.5–2.0 and 1.3–1.6 respectively), while duration, total distance, and HIE showed small, likely substantial effects. PlayerLoad™ and total distance had likely substantial effects on 30-m sprint time (small increases of 2.1–2.6%). Effects on countermovement jump performance were generally non-substantial. Despite these relationships, the uncertainty was too large to predict the recovery of individual players from the external load variables.

Conclusions: This study provides evidence that external load variables have an effect on recovery markers up to 72 h post-match. Hence, tracking external load in matches may be helpful for practitioners when managing training load and recovery strategies post-match. However, it is recommended that several different external load variables are monitored. Future research should continue to address the problem of predicting recovery from external load variables.

Keywords: neuromuscular fatigue, muscle damage, performance, sprint, player monitoring

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INTRODUCTION

Football match load is known to cause increases in muscle damage indicators (Andersson et al., 2008), alter the biochemical milieu (Ascensão et al., 2008), and cause glycogen depletion (Bangsbo et al., 2006), leading to neuromuscular fatigue and physical performance impairment up to 72–96 h post-match (Silva et al., 2018a). In this rather long post-match period, information on the players' recovery status could be useful in order to optimally manage training load and recovery strategies for the individual player. Measuring the recovery status directly is however time-consuming and often involves invasive measurements or performance tests that are difficult to implement in the daily training routine. Conversely, the use of player tracking technology to measure external load in training and matches is easy and requires minimal player involvement and additional assessments. The use of such technology in team sports has escalated in recent years, both in research (Malone et al., 2017) and in practical applications (Akenhead and Nassis, 2016). Global Navigation Satellite Systems (GNSS) and Local Positioning Systems (LPS) with integrated Inertial Measurements Units (IMU) provide data on position, distance, speed, and accelerative efforts as measures of external load. While shown to have good reliability (Luteberget et al., 2018a) and validity (Luteberget et al., 2018b), player tracking systems have limited value if the quantified external load is not related to performance, fatigue, or recovery.

A few studies have investigated the relationship between external load variables and recovery from football matches *via* muscle damage indicators in blood and neuromuscular fatigue measurements (Thorpe and Sunderland, 2012; de Hoyo et al., 2016; Russell et al., 2016; da Silva et al., 2018b). While these studies have reported associations between creatine kinase (CK) and high-intensity running distance, sprint distance, and number of sprints, between myoglobin (MYO) and number of sprints, and between countermovement jump performance (CMJ) and decelerations and accelerations, they are somewhat limited to correlation analyses with small sample sizes. Furthermore, from a practical point of view, there are a lack of studies investigating the specific effect of external load variables on recovery markers, both the magnitude of the effect and the recovery time back to baseline values. One exception is Rowell et al. (2017) who found a dose-response relationship of PlayerLoad™ on CMJ, but only one external load variable was investigated. Consequently, studies investigating several external load variables and also their effect on important physical performance parameters such as sprint or intermittent running performance are needed.

Seventy-two hours post-match is a key time-point where the next match or a hard training session may take place. Most studies have examined the relationships for only 24–48 h post-match (Thorpe and Sunderland, 2012; de Hoyo et al., 2016; Russell et al., 2016; da Silva et al., 2018b), despite evidence showing substantial changes in recovery markers at 72 h post-match (Ascensão et al., 2008; Ispirlidis et al., 2008). Additionally, due to individual differences in recovery time, some players might be recovered and some players not, hence being able to predict the recovery status on day three post-match is practically important.

The objective of the current study was therefore to investigate how external load affects recovery up to 72 h after a football match. External load was quantified as playing duration, high-intensity events (HIEs), high-intensity running distance (HIR), PlayerLoad™, and total distance covered. Recovery was operationalized into recovery markers for muscle damage indicators (CK and myoglobin, MYO); neuromuscular function (countermovement jump, CMJ); sprint performance (30-m sprint, SP30); and intermittent endurance performance (Yo-Yo Intermittent Recovery test level 1, YOYO). A secondary objective was to investigate how different amounts of external load affect the recovery status 72 h post-match.

MATERIALS AND METHODS

Participants

Seventy-five outfield male football players from six Norwegian second division teams participated in the study, of whom subject characteristics are summarized in **Table 1**. The players reported an average of 7.6 ± 2.3 training sessions per week (matches excluded) for a typically in-season week, with 80% of the players reporting "less" or "somewhat less" training load in the last week before their experimental match. The number of players included in the different analyses is highlighted in **Figure 1**.

Study Design

The study took place 14–23 days after their last match of the season. It consisted of three experimental matches (one per team) with corresponding familiarization-, pre- and post-match tests conducted at −144, −72, −1, 1, 24, 48, and 72 h relative to the matches. When conducted on the same day, the test order was: blood samples, CMJ, SP30, and YOYO. The players were instructed to refrain from other intense physical exercises within the study period and to follow their normal preparation before the match regarding nutrition and sleeping strategies. The matches were preceded by a standardized 40-min warm-up consisting of 5 min of jogging, the CMJ test procedure,

TABLE 1 | Summary of subject characteristics and baseline values for recovery markers.

Characteristic	*n*	Mean	SD	Min	Max
Subject characteristics					
Age (yr)	75	20.4	4.6	16	45
Height (cm)	75	178.0	6.1	164	194
Body mass (kg)	75	72.7	7.2	49	96
Baseline values					
CK (U/L)	49	367	273	59	1,600
MYO (μg/L)	49	39	37	21	256
CMJ (cm)	59	43.0	4.5	33.2	57.5
SP30 (s)	32	4.27	0.18	3.62	4.53
YOYO (m)	24	2,000	388	1,200	2,800

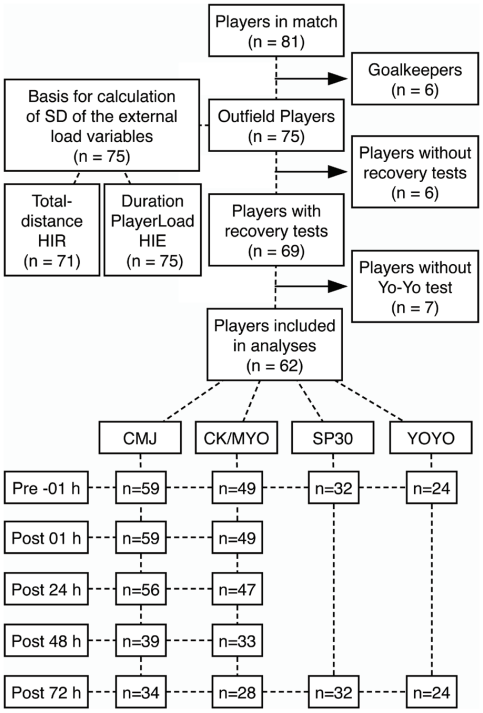

FIGURE 1 | Flow chart showing number of participants included in (1) the analyses of the different recovery markers on each time-point and (2) the calculation of SD which were used for the rescaling of the external load variables.

team-organized running drills, and a play exercise. Standard 90-min matches were officiated according to FIFA rules, and teams and players were instructed to give their best to win. Immediately after the match, the players consumed a 330-ml recovery drink (Yt Restitusjonsdrikk, TINE, Norway). In accordance with the study objectives and typical substitution practices in official matches, two to three pre-planned substitutions at 45 and 60 min were implemented per team to spread the match load from low to high values.

Recovery Testing Procedures

Venous blood samples were drawn, centrifuged at 4°C for 10 min at 1300 g, and stored in −80° until analyzed for CK and MYO at the Oslo University Hospital, Rikshospitalet (Oslo, Norway; Cobas 8,000, Roche Diagnostics, USA). The laboratory's stated coefficient of variation (CV) is 5 and 6% for CK and MYO respectively. Baseline values were taken from the −1-h blood sample.

CMJ, with hands placed on hips, was performed on a portable force platform (FP4, HUR labs, Tampere, Finland) and jump height was analyzed by the provided software (Force Platform Software Suite, Version 2.6.51, Kokkola, Finland). Data from our lab show a CV of 4.7%. The warm-up procedure consisted of a 5-min jog followed by three jumps with 80, 90, and 100% effort. Each player performed three to five jumps, interspersed with 15 s of rest, where the highest jump was used for analyses. The best of the −72- and −1-h CMJ was used as the baseline value.

SP30 was conducted with error correction processing timing gates (SmartSpeed Pro, Fusion Sport, Brisbane, Australia) placed at 0 and 30 m, and with a starting position 0.3 m before the first gate. Participants were instructed to start in a static, forward leaning position, and then sprint as fast as possible past a cone placed at 35 m. The best of three trials, with minimum 2-min rest between, was exported for analysis. Reliability testing from our lab shows a CV of 1.7%. Baseline values were taken from the −72-h SP30 test.

The YOYO test was conducted according to the instructions described by Krustrup et al. (Krustrup et al., 2003). A specific warm-up consisting of the 11 first stages of the test were undertaken, followed by a 2-min rest. The total distance in meters was used in the analysis. Furthermore, the best of the pre- and post-results (YOYOmax) was used as a measure of the players' aerobic fitness. The test-retest CV is shown to be 4.9% (Krustrup et al., 2003). Baseline values were taken from the −72-h YOYO test.

Tracking of External Load

All three matches were played in the same indoor football stadium (105 m by 65 m) with a third-generation artificial turf, temperature of 15 ± 1°C, and a relative humidity of 77 ± 5%. The players wore two different tracking devices, one IMU device (OptimeEye S5, Catapult Sports, Australia, with GNSS turned off) and one LPS device (ClearSky T5, Catapult Sports, Australia). These devices were taped together, with the IMU closest to the body and located between the scapulae in a manufacturer-provided vest (Catapult Sports, Australia). All IMU devices were calibrated according to the manufacturer's instructions. The LPS was set up with 18 anchor nodes fixed around the pitch, and spatial calibration was carried out according to manufacturer's recommendations. Three players missed LPS data due to signal problems and one due to limited available LPS devices.

Data Processing

Five different external load variables were chosen to provide different representations of the actual match load. Playing duration (on field time), PlayerLoad™, and HIE were extracted from the IMU device using the Sprint software (version 5.1.7, Catapult Sports, Australia), and total distance and HIR were extracted from the LPS using the Openfield Software (version 1.12, Catapult Sports, Australia). PlayerLoad™ is a vector magnitude expressed in arbitrary units as the square root of the sum of the squared instantaneous rate of change in acceleration in three dimensions, described more comprehensively by Boyd et al. (2011). HIE is the sum of acceleration, deceleration, and change of direction events exceeding a threshold of 2.5 m/s based on procedures by Luteberget and Spencer (Luteberget and Spencer, 2017). During indoor field assessment, HIE and PlayerLoad™ have shown an inter-device CV of 3.1 and 0.9%

respectively (Luteberget et al., 2018a). HIR is the total distance covered with running speed over 19.8 km/h, while total distance is the total distance covered at any speed. A validity study using the same LPS system as the current study has shown a 2–4% error in linear and nonlinear distance when conducted in an indoor environment (Sathyan et al., 2011).

Statistical Analysis

The recovery markers were log-transformed, to reduce bias due to nonuniformity of errors, before being analyzed as change-scores using a linear mixed model (MIXED procedure in SAS 9.4 Software; SAS Institute, Cary, NC, USA). The effects were back-transformed to express factor or percent changes. Time, Time × external load variable, Time × baseline, and Time × YOYOmax were specified as fixed effects, with Time treated as nominal variable. When YOYO was the dependent variable, YOYOmax was omitted from the model because it contained partly the same numbers as YOYO baseline. To deal with interdependency and unequal variances in the models with repeated measurements (CK, MYO, and CMJ), the R matrix were specified with Time, PlayerID as blocks and an "unstructured" covariance structure, using the REPEATED statement in SAS. SP30 and YOYO had no repeated measurements and were analyzed without a REPEATED statement. Separate analyses were done for each external load variable for every recovery marker. The main effect of interest, Time × match load, was adjusted for baseline to address the regression to the mean effect, and YOYOmax to address the possibility of fitness being a confounder affecting both match load (Krustrup et al., 2003; Bradley et al., 2013; Redkva et al., 2018) and recovery (Johnston et al., 2015). Furthermore, to properly evaluate the magnitude of the effect of continuous variables, they were rescaled by dividing by two standard deviations (SDs). Two SDs also correspond approximately to the mean separation of lower and upper tertiles (Hopkins et al., 2009), and can be justified as a separation of typically high and low match loads. The magnitude of the effects is presented as standardized effect sizes (ES: the effects divided by the SD of the baseline value), where <0.2, 0.2–0.6, 0.6–1.2, 1.2–2.0, and >2.0 are regarded as trivial, small, moderate, large, and very large effects respectively. Nonclinical, magnitude-based inferences were used, where an effect was deemed unclear if the 90% confidence interval included small positive and negative effects; the effect was otherwise deemed clear. Qualitative assessment of chances of clear outcomes were as follows: >25–75%, possibly; >75–95%, likely; >95–99%, very likely; >99%, most likely (Hopkins et al., 2009).

RESULTS

Match Load

As a result of substitutions, the match load across all players was spread in a linear manner for all external load variables, except for duration where 61% of the players played a full 90-min match. Descriptive summaries of total and relative match load are shown in **Table 2**.

Mean Change in Recovery Markers

Baseline values of the recovery markers are shown in **Table 1**, and the mean changes in recovery markers from pre- to 1, 24, 48, and 72 h post-match are presented in **Figure 2**. The matches induced most likely substantial increases in CK at 1 h (ES = 0.92), 24 h (ES = 1.20), and 48 h (ES = 0.67) post-match, whereas a likely substantial increase was seen 72 h post-match (ES = 0.32). Myoglobin peaked at 1 h post-match with a most likely substantial increase (ES = 3.80), followed by a most likely substantial increase at 24 h (ES = 0.78), and possibly substantial increases at 48 h (ES = 0.27) and 72 h (ES = 0.30). CMJ height showed a most likely substantial decrease at 1, 24, and 48 h and a likely substantial decrease at 72 h post-match with ES of −0.75, −0.68, −0.68, and −0.25 respectively. SP30 showed a likely substantial increase (ES = 0.38) at 72 h post-match, while for YOYO, the effect was trivial and unlikely substantially positive (ES = −0.08).

The Effects of External Load Variables on Recovery Markers

The effects of the external load variables on recovery markers at each time-point are presented in **Figure 3**. The external

TABLE 2 | Summary of total match load and match load per minute for selected external load variables, for all players and for different groups of players (mean ± SD).

Group	*n*	Duration (min)	Distance (m)	PlayerLoad™ (AU)	HIE (#)	HIR (m)
Total match load						
All	75	72.7 ± 24.9	8,305 ± 2,627	780 ± 290	152 ± 62	380 ± 200
Entire match	44	91.2 ± 1.0	10,110 ± 972	966 ± 174	185 ± 52	434 ± 199
Replaced	16	54.7 ± 16.8	6,673 ± 2,016	637 ± 191	124 ± 43	357 ± 205
Substitute	15	37.4 ± 13.7	4,483 ± 1,075	386 ± 123	85 ± 31	237 ± 113
Match load per min						
All	75		116 ± 14	10.8 ± 1.8	2.2 ± 0.6	5.4 ± 2.6
Attackers	10		112 ± 7	10.0 ± 1.2	2.1 ± 0.4	5.0 ± 2.3
Central defenders	14		101 ± 5	9.1 ± 1.0	1.7 ± 0.5	3.2 ± 1.3
Central midfielders	22		128 ± 12	12.2 ± 1.9	2.4 ± 0.5	5.0 ± 2.3
Fullbacks	13		112 ± 14	10.6 ± 1.0	2.0 ± 0.5	6.0 ± 1.6
Wide midfielders	16		117 ± 12	11.3 ± 1.5	2.4 ± 0.5	8.0 ± 2.7

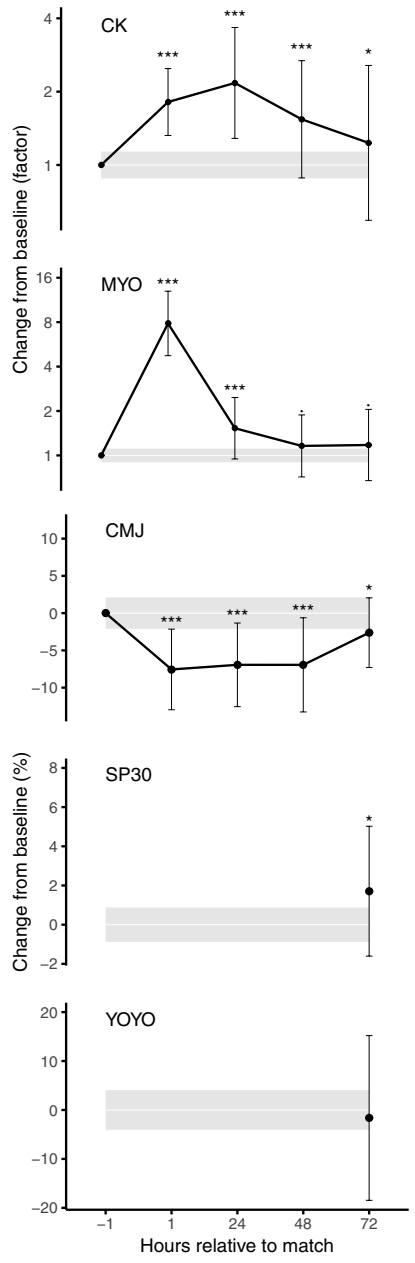

FIGURE 2 | Factor or percent change from baseline for the specific recovery markers in the hours post-match, adjusted for baseline, PlayerLoad™, and YOYOmax. The spread is indicated by SD and shaded area represents trivial changes. Probabilistic statements about the true effect are labeled as follows: · = possibly, * = likely, and *** = most likely.

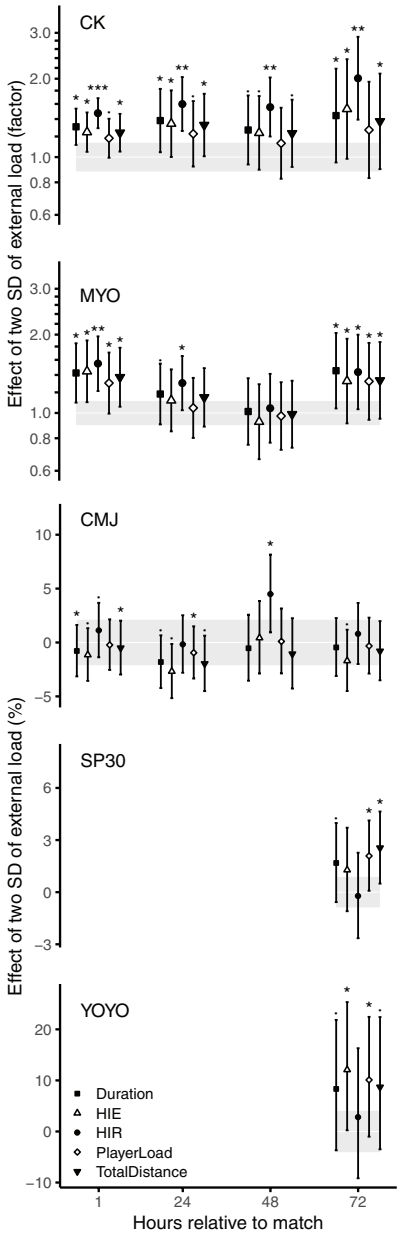

FIGURE 3 | The factor or percent effect of two SDs of match load on recovery markers at the specific time-points, adjusted for baseline and YOYOmax. Two SDs of match load are interpreted as the difference between matches with typical high and low load. Uncertainty in the estimates is indicated by 90% confidence intervals and shaded area represents trivial changes. Probabilistic statements about the true effect are labeled as follows: · = possibly, * = likely, ** = very likely, and *** = most likely.

load variables had positive effects on the muscle damage indicators. HIR had the strongest relationship with CK showing very likely to most likely substantial effects, consistent throughout all time-points (ES = 0.60–1.08). Duration, total distance, and HIE showed likely substantial effects on CK at 1 h (ES = 0.33–0.42), 24 h (ES = 0.44–0.50), and 72 h (ES = 0.49–0.66). The effects on MYO at 1 h post-match was very likely substantial for HIR (ES = 0.80) and likely substantial for duration (ES = 0.65), HIE (ES = 0.68), total distance (ES = 0.58), and PlayerLoad™ (ES = 0.49). Except for a likely substantial increase of HIR (ES = 0.49) and a possibly substantial effect of Duration (ES = 0.31) at 24 h, the other effects at 24 h and 48 h post-match were unclear. At 72 h, likely substantial effects on MYO were found for all variables (ES = 0.52–0.69). The observed effects on CMJ were generally trivial or unclear, except for a possibly substantial negative effect of HIE at 24 h (ES = −0.26) and a likely substantially positive effect of HIR at 48 h post-match (ES = 0.40). SP30 performance 72 h post-match was affected negatively by total distance (ES = 0.56) and PlayerLoad™ (ES = 0.46), showing likely substantially negative effects. On the contrary, likely substantially positive effects of HIE (ES = 0.56) and PlayerLoad™ (0.47) were seen on YOYO performance 72 h post-match.

Effect of External Load Variables on Recovery Status 72 h Post-match

The predicted mean changes in recovery markers at 72 h for given match loads are depicted in **Figure 4**. External load variables that are substantially affecting recovery markers are highlighted in **Figure 3**. Other external load variables were non-substantial meaning that a change in match load could cause either trivial change, or substantial increase or decrease in the recovery markers. While substantial effects were seen on predicted means for some external load variables, prediction intervals for individual values covered both substantially negative and substantially positive values throughout the range of match load on all external load variables.

DISCUSSION

This study investigated how external load variables, derived from player tracking devices, affected subsequent recovery up to 72 h post-match. The external load variables were found to impact both the magnitude and the length of the recovery. HIR was the strongest predictor of muscle damage indicators, while PlayerLoad™ and total distance predicted recovery of sprint performance, and HIE and PlayerLoad™ predicted YOYO performance. Unexpectedly, recovery of CMJ performance could not be predicted. Despite these substantial mean effects, external load variables were not able to predict recovery in individual players.

Impact on Muscle Damage Indicators

The increases observed in CK and MYO post-match indicates muscle damage which could be categorized as mild exercise-induced muscle damage (Paulsen et al., 2012). The response is comparable to other studies with reserve teams (Thorpe and Sunderland, 2012; Russell et al., 2016) and professional players (Silva et al., 2013), despite that the mean match duration, total- and high-intensity distance were lower than observed in a typical full match (Bradley et al., 2013). Furthermore, the response was higher than reported by de Hoyo et al. (2016), who also included substitutes in their analysis. These comparisons suggest a high response of muscle damage indicators in the current study. Following the same patterns as in a recent meta-analysis (Silva et al., 2018a), CK and MYO peaked at 24 and 1 h post-match, respectively, and an increase from baseline was still evident after 72 h for CK. Large variations were observed at 72 h, meaning that the muscle damage indicators had returned to baseline in some players, but not in others. For example, two players still had increasing CK at 72 h to over 3,200 $U^{*}L^{-1}$, suggesting a more severe muscle damage (Paulsen et al., 2012).

This is the first study modeling the effect of different external load variables on recovery markers, for a full 72-h time period post-match in football players. The effects, understood as the difference between a typical high and low match load, provide evidence that match load explains changes in the two indicators of muscle damage (**Figure 3**). Of the five external load variables, HIR was the strongest predictor, consistent throughout all time-points. The larger effect of HIR is supported by other studies where change in CK correlated with high-intensity distance and number of sprints, but not for total distance (Thorpe and Sunderland, 2012; de Hoyo et al., 2016; Russell et al., 2016). The reason for the larger effect could be the high-force and high-speed muscle contractions occurring when maintaining or decelerating from high running speeds, causing muscles to work while lengthening. Such eccentric muscle contractions are shown to cause tearing and disruption of muscle fibers (Paulsen et al., 2012). HIE and PlayerLoad™, that are based on accelerometer data, could hypothetically assess football-specific movements such as accelerations, decelerations, and change of directions to a higher degree than for example distance covered. Instead, our data show that HIE had a lower effect than HIR on CK and MYO, suggesting that running speed is an important factor for muscle damage. PlayerLoad™ on the other hand had the lowest effects which makes it a poor predictor of muscle damage indicators.

Impact on Neuromuscular Fatigue

The observed decrease in CMJ performance suggests a neuromuscular fatigue comparable to other studies (Nedelec et al., 2014). Unexpectedly, the decrease in CMJ could not be explained by any of the external load variables in contrast to Rowell et al. (2017) where a dose-response relationship was found between low, medium, and high PlayerLoad™ groups and CMJ height 0.5 and 18 h post-match. Moreover, Russell et al. (2016) found moderate correlations between change in peak power output from CMJ and high-intensity running distance and sprint distance.

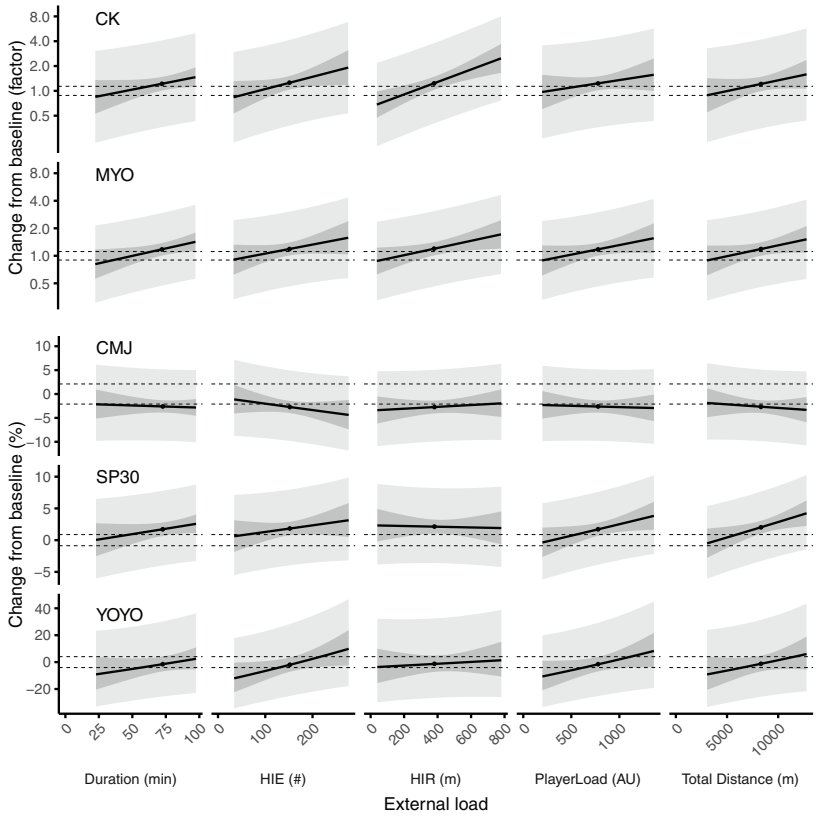

FIGURE 4 | Predicted mean change in recovery markers at 72 h post-match for different amounts of match load, after adjustments for baseline and YOYOmax. Match load values are within the range of match load values in this study and mean match load is indicated by a dot symbol. The area between the dotted lines indicates trivial changes; the dark shaded area shows 90% confidence interval, and the light shaded area shows 90% prediction interval.

Other studies have found short-lived relationships between change in CMJ and high-intensity accelerations (Russell et al., 2016), hard changes of directions (Nedelec et al., 2014) at 24 h, and decelerations at 0.5 h and at 48 h (de Hoyo et al., 2016). These relationships suggest that CMJ performance could be linked to accelerative efforts that target the same muscles that are active in CMJ. Although we did find a possibly small effect of HIE on CMJ at 24 h, the uncertainty in the estimates and inconsistency over the time-points does not provide strong evidence for such relationship. Hence, one might also question if these specific variables really are able to identify the true match load that causes neuromuscular fatigue.

Impact on Sprint and Intermittent Endurance Performance

The decreased SP30 performance at 72 h post-match indicates that sprint performance is not recovered 3 days post-match, in line with some studies (Ispirlidis et al., 2008; Fatouros et al., 2010), but not all (Silva et al., 2013). PlayerLoad™ and total distance showed small effects on SP30 at 72 h. To our knowledge, no other studies have examined such relationship. As opposed to muscle damage, which was affected by high-intensity work, SP30 was affected by variables describing match load volume. In line with this finding, it has been proposed that recovery of sprint performance could be linked to the duration of exercise, as basketball and handball have shown shorter recovery times than football (Doeven et al., 2018). For YOYO, no substantial change was found from baseline to 72 h post-match. Nevertheless, positive effects of HIE and PlayerLoad™ were still found, suggesting that higher match load improves the YOYO performance 72 h. The reason could be that a conditioning effect, due to that the match was played a couple of weeks after the season, was evident for the players with the highest match load, while not in the players with the lowest match load. Such conditioning effect could be explained by fitter players perform more running activity (Krustrup et al., 2003), but also recover faster (Johnston et al., 2015).

Match Load As a Predictor of Recovery Status

The substantial effects of external load variables on CK, MYO, and SP30 that were seen at 72 h post-match provide evidence that match load affects the time to recovery. Thus, players with low match load could recover at 72 h, while players with high match load could not. Such a finding has important practical applications for teams using tracking devices when managing recovery strategies or training load. Moreover, our data showed that some external load variables could predict recovery on average, but not in individuals based on the wide prediction interval (**Figure 4**). The wide prediction intervals seen in **Figure 4** are a consequence of large individual differences in the recovery, as indicated by the SD in **Figure 2**. Some of the variability in the recovery might be explained by differences in the individual player's relative match load, i.e., the current match load compared to his typical match load over several matches. Given the large within-player, match-to-match variation in external load seen in football (Carling et al., 2016; Al Haddad et al., 2018), some players had presumably a higher relative external load, while others had lower relative load. A multiple-match design must be carried out to address if differences in within-player external load could predict the recovery from match more reliably than between-player external load.

LIMITATIONS

A limitation of the study is the aforementioned one match per player design, which only allowed for between-player modeling of the external load variables. A multiple-match design that models the effect of within-player match load could possibly have decreased the uncertainty in the estimates of recovery. In addition, we suspect that the study, especially on the last two time-points, was somewhat underpowered as some of the measures were inherently unreliable. Although the external load and recovery data were regarded as representative, the matches were nonofficial matches, played 2–3 weeks after the season, in a period without other matches and with a lower self-reported training load. Hence, the match load and the recovery from the match might have been different from an official, within-season match. Lastly, the control of the players' physical activity, nutrition strategies, and sleep before and after the match were limited to pre-study instructions from the research staff.

CONCLUSIONS AND PRACTICAL APPLICATIONS

This study provides evidence that external load variables derived from player tracking systems have an effect on recovery markers up to 72 h post-matches. Such information may help practitioners when managing training load and recovery strategies post-match. HIR had the most substantial effect on muscle damage indicators, and PlayerLoad™ and total distance affected sprint performance. Hence, a combination of several different tracking device variables is advised to ensure a better representation of the match load. An unexpected finding, which requires further investigation, was the trivial effect of external load variables on CMJ. While the mean changes in recovery markers approached baseline values at 72 h post-match, the effects of external load variables on the same recovery markers were still substantial, suggesting that external load variables could partly explain the time to recovery. Despite these substantial effects, it was not possible to predict the recovery of individual players at 72 h from any of the external load variables due to too much uncertainty in the predictions.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of Regional Committees for Medicine and Health Research Ethics. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The study's data storage methods were approved by the Norwegian Centre for Research Data.

AUTHOR CONTRIBUTIONS

HW, TR, and MS were responsible for the idea and the design of the study. Data were collected by HW, LL, II, and MS. HW was responsible for the data analyses and drafted the manuscript. All authors contributed to the interpretation of the results, critical revision, and approval of the final version.

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Paper III

Muscular heat shock protein response and muscle damage after semi-professional football match

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ORIGINAL ARTICLE

WILEY

Muscular heat shock protein response and muscle damage after semi-professional football match

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Purpose: A typical football match leads to neuromuscular fatigue and physical performance impairments up to 72–96 h post-match. While muscle damage is thought to be a major factor, damage on the ultrastructural level has never been documented. The purpose of this study was to investigate post-match cellular muscle damage by quantifying the heat shock protein (HSP) response as a proxy for protein damage.

Methods: Muscle biopsies, blood samples, countermovement jumps, and perception of muscle soreness were obtained from twelve semi-professional football players 1, 24, 48, and 72 h after a 90-min football match. Muscle biopsies were analyzed for αB-crystallin and HSP70 in the cytosolic and cytoskeletal sub-cellular fractions by Western blotting. Fiber type-specific αB-crystallin and HSP70 staining intensity, and tenascin-C immunoreactivity were analyzed with immunohistochemistry. Blood samples were analyzed for creatine kinase and myoglobin.

Results: Within 24 h post-match, a 2.7- and 9.9-fold increase in creatine kinase and myoglobin were observed, countermovement jump performance decreased by −9.7% and muscle soreness increased by 0.68 units. αB-crystallin and HSP70 accumulated in cytoskeletal structures evident by a 3.6- and 1.8-fold increase in the cytoskeletal fraction and a parallel decrease in the cytosolic fraction. In type I and II fibers, αB-crystallin staining intensity increased by 15%–41% and remained elevated at 72 h post-match. Lastly, the percentage of fibers with granular staining of αB-crystallin increased 2.2-fold.

Conclusions: Football match play induced a muscular HSP stress response 1–72 h post-match. Specifically, the accumulation of HSPs in cytoskeletal structures and the granular staining of αB-crystallin suggests occurrence of ultrastructural damage. The damage, indicated by the HSP response, might be one reason for the typically 72 h decrease in force-generating capacity after football matches.

KEYWORDS

exercise-induced muscle damage, match load, neuromuscular fatigue, recovery, soccer

1 | INTRODUCTION

A typical football match leads to neuromuscular fatigue and physical performance impairments lasting up to 72–96 h post-match.[1] Considering that elite players play 1–2 matches per week during a 9–10 month long season, this has major implications on the weekly training routine and preparations for the upcoming matches. The post-match fatigue is thought to be caused by dehydration, glycogen depletion, mental fatigue, and muscle damage, where muscle damage is likely a major factor.[2] The evidence for muscle damage is based on large increases in indirect markers for muscle damage such as blood concentrations of creatine kinase and myoglobin,[3,4] increases in delayed onset of muscle soreness,[5,6] swelling,[5] and reduction in force-generating capacity[7] and power.[3] However, studies from football matches documenting muscle damage at a cellular level are lacking and may increase the understanding of mechanisms behind the long recovery period after football matches.

Exercise-induced muscle damage is typically caused by unaccustomed muscle work, excessive force production, overstretching, and eccentric muscle actions.[8] It is characterized by a decreased force-generating capacity, increase in muscle soreness, tissue swelling, and increases of muscle proteins in the blood such as creatine kinase and myoglobin.[9] At the cellular level, ultrastructural damage is characterized by cellular and sub-cellular disturbance, observed typically as z-line streaming[10] and sarcomere disruptions[11] on high magnification electron micrographs. Co-localized on the damaged myofibrils is heat shock proteins (HSP),[12] a family of highly conserved proteins which functions as chaperones, helping to stabilize and refold damaged proteins.

Two of the commonly studied HSPs are αB-crystallin and HSP70. αB-crystallin is one of the small HSP (22 kDa) and seems to bind to Z-disc-related structures, possibly to the intermediate filament protein desmin, after muscle-damaging exercise.[13] The αB-crystallin response is rapid and can be observed within 0.5 h post-exercise.[14] HSP70, which seems to be more involved in refolding and degradation of damaged proteins,[15] often has a more delayed and sustained response.[14] Interestingly, exercise-induced muscle damage seems to lead to a translocation of the HSP from a soluble, unbound state in the cytosol, to binding to stressed structure of the cytoskeleton and sarcomeres.[12,13,16] After Western blotting, this is evident as a reduction in the amount of HSP in the cytosolic fraction and an concomitant increase in the cytoskeletal fraction. Furthermore, accumulation of the small HSP at disrupted sarcomeres has been observed via both electron and fluorescence microscopy, in the latter often as granular staining.[12] Hence, the HSP response to exercise can be regarded as a proxy measure for ultrastructural muscle damage.

Exercise may also induce muscle damage to extracellular structures. The extracellular matrix protein tenascin-c, which has de-adhesive function in remodeling of the extracellular matrix after muscle injury,[17] is rapidly upregulated in the endomysium after increased loading on skeletal muscles[18,19] and has been suggested as an indicator of disruptions in the extracellular matrix.[11,20] Tenascin-c could therefore be a marker for remodeling of extracellular matrix and should increase rapidly after damaging exercise.

Muscle damage has been suggested as a central mechanism of neuromuscular fatigue after football matches,[1,2] but to what extent cellular damage occurs to muscle fibers after football matches is not known. Hence, the aims of the current study were to (1) quantify the HSP response, as a proxy for muscle damage, in the cytosolic and cytoskeletal sub-cellular fractions and identify potential translocation; (2) compare the HSP response in type I vs type II muscle fibers; (3) discuss the HSP response in relation to systemic markers of muscle damage such as creatine kinase, myoglobin, loss in power, muscle soreness, and match load.

2 | MATERIALS AND METHODS

2.1 | Participants

This study was a part of a large study with 81 male semi-professional football players from six clubs competing in the third highest national league in Norway.[4] A subset of twelve players (mean ± SD; age 19.4 ± 2.3 y, height 175 ± 6 cm and body mass 71 ± 6 kg) from five of the teams, 1–3 from each team, volunteered to donate muscle biopsies. The group of players consisted of 2 attackers, 2 central defenders, 4 central midfielders, 3 full backs, and 1 wide midfielder. The self-reported number of training sessions for a typical in-season training week was 8 ± 2. In the last week before their experimental match, 50% of the players reported to have "somewhat less" and 25% reported "less" training sessions than a typical in-season week. The players' physical fitness was tested 72 h pre-match with Countermovement jump (CMJ; 42.5 ± 2.3 cm), 30-m sprint (4.23 ± 0.11 s) and the Yo-Yo intermittent recovery test level 1 (2003 ± 282 m), a football specific shuttle run test that stresses both the aerobic and anaerobic energy systems.[21] All players were informed about potential risks and gave written informed consent before commencing the study. The study was approved by the Regional Ethics Committee of South-East Norway (2015/1869).

2.2 | Experimental design

The study took place 14–23 days after their last match of the season. The six club teams were set up by the investigators to form three experimental matches (one match per team), where the opponents were considered as rivals. All matches were played in the same indoor football stadium (105 m by 65 m), covered with a 3rd generation artificial turf, a turf that was common for all players. The baseline and post-match muscle biopsies, blood samples, CMJ, and perception of muscle soreness were obtained −1, 1, 24, 48, and 72 h relative to the matches, except for the baseline muscle biopsies which were obtained 3 to 6 days before the match. The sample size at each time point for the different outcome measures are presented in Table 1. The players were instructed to refrain from other intense physical exercise within the study period and to follow their normal preparation before the match regarding nutrition- and sleeping strategies. The matches were preceded by a standardized 40-min warm-up consisting of 5-min low-intensity jogging, the CMJ test procedure, team organized running drills, and a small-sided game exercise. Standard 90-min matches were refereed by official referees according to FIFA rules, and teams and players were instructed to give their best to win. Immediately after the match, the players consumed a 330 ml recovery drink (Yt Restitusjonsdrikk, TINE, Norway) containing 30 g carbohydrate, 20 g protein, and 3.5 g fat.

2.3 | External match load, blood sampling, CMJ, and muscle soreness

The players' external match load, including total distance covered (m), high-speed running distance (m > 5.5 m/s), and PlayerLoad^{TM} (AU)[22] were assessed by two tracking systems (OptimeEye S5 and ClearSky T5, Catapult Sports, Australia) described more extensively in Wiig et al.[4] Venous blood samples were drawn, centrifuged at 4°C for 10 min at 1300 g and stored at −80°C until analyzed for creatine kinase and myoglobin (Cobas 8000 c702/e602, Roche Diagnostics, Basel, Switzerland). CMJ, with the hands placed on the hips, was performed on a portable force platform (FP4, HUR labs, Tampere, Finland), and jump height was analyzed by the provided software (Force Platform Software Suite, Version 2.6.51, Kokkola, Finland). Data from our laboratory showed a coefficient of variation of 4.7%. The warm-up procedure consisted of a 5-min jog followed by three jumps with 80%, 90%, and 100% effort. Each player performed three to five jumps (until no further improvement), interspersed with 15 s rest, the highest jump was used for analyses. The best of the −72 and −1 h CMJ was used as the baseline value. General muscle soreness was assessed by rating their perceived soreness on an ordinal scale from 1 to 5, corresponding to very sore, increase in soreness/tightness, normal, feeling good, feeling great. Soreness registration was undertaken in the morning via a mobile app (PMSYS, University of Oslo, Oslo, Norway).

2.4 | Muscle sampling

Muscle biopsies were obtained from the mid-portion of *m. vastus lateralis* from their dominant leg (baseline, 1 and 72 h time points), and from their non-dominant leg (24 and 48-h time points). The insertions of the repeated biopsies were placed 3 cm proximally from the previous biopsy to minimize any impact of the procedure itself on the muscle samples. The procedure was performed under local anesthesia (Xylocain adrenalin, 10 × mg/ml + 5 × μg/ml; AstraZeneca, London, UK), and approximately 200 mg (2–3 × 50–150 mg) of muscle tissue was obtained with a modified Bergström needle using the suction

TABLE 1 Sample size for each outcome measure and time point (baseline $n = 12$). Several biopsy analyses were excluded due to poor muscle tissue quality while some players omitted the biopsy procedure at 48 h

Outcome variable	Structure	1 h	24 h	48 h	72 h
αB-crystallin	Cytosolic	12	12	7	11
αB-crystallin	Cytoskeletal	11	10		9
αB-crystallin	Type I & II	12	12	7	11
HSP70	Cytosolic	12	12	7	11
HSP70	Cytoskeletal	12	12		11
HSP70	Type I & II	12	12		11
Granular αB-crystallin		10	11		9
Tenascin-C		12	12		11
Creatine kinase & myoglobin		11	11	10	11
CMJ		11	11	9	11
Muscle soreness			11	8	

technique. The portion of muscle tissue used for homogenization was quickly rinsed in physiological saline before visible fat, connective tissue, and blood were removed. The sample weight was recorded before the tissue was frozen in dry-ice-cooled isopentane. A separate muscle tissue sample, for immunohistochemistry, was mounted in OCT embedding matrix (KMA-0110-00A, CellPath, Newtown Powys, UK) and quickly frozen in isopentane, pre-cooled on liquid nitrogen to the freezing point. All muscle samples were stored at −80°C until further analyses.

2.5 | Homogenization and Western Blot procedures

Approximately 50 mg of muscle tissue was homogenized and fractionated into cytosolic, nuclear, membrane, and cytoskeletal fractions using a commercial fractionation kit (ProteoExtract Subcellular Proteome Extraction Kit, 539790, Calbiochem, EMD Millipore Corporation, Billerica MA, USA). Protein concentration was measured using a commercial kit (Bio-Rad DC protein assay, 0113, 0114, 0115; Bio-Rad Laboratories, Inc., Hercules, CA, USA) and measured by colorimetric intensity using a filter photometer (Expert 96, ASYS Hitech GmbH, Ec, Austria). Protein concentration was calculated using Kim32 software (Kim Version 5.45.0.1, Dan Kittrich, Prague, Czech Republic).

The cytosolic and cytoskeletal fractions were analyzed by Western blotting. Between 6 and 24 μg of denatured proteins, depending on the sub-cellular fraction, were separated by electrophoresis through 4%–20% gradient gels (Mini-PROTEAN® Stain-FreeTM Gels, 456–8094, Bio-Rad Laboratories, Inc.) under denaturing conditions at 200 volts for 30 min in running buffer (10x TGS Buffer, 161–0732; Bio-Rad Laboratories GmbH, München). Proteins were then transferred to PVDF membranes (Immun-Blot PVDF, 162–0177; Bio-Rad Laboratories, Inc.), which were immersed in a blocking solution consisting of 5% fat-free skimmed milk in TBS with 0.1% Tween-20 (TBS-T) (TBS, 1706435, Bio-Rad Laboratories, Inc.; Tween-20, 437082Q, VWR International, Radnor, PS, USA; skim milk powder 1.15363, Merck KGaA, Darmstadt, Germany) for 2 h at room temperature. Blocked membranes were incubated with primary antibodies (Table 2) against αB-crystallin or HSP70 (Enzo Life Sciences, Inc., Farmingdale, NY, USA) over night at 4°C with gentle agitation. Incubation with horseradish peroxidase-coupled secondary antibodies (Goat anti-Mouse IgG Thermo Fisher Scientific, Inc., Hanover Park, IL, USA) was done for 1 h at room temperature with gentle agitation. All antibodies were diluted in TBS-T with 1% fat-free skimmed milk. Between stages, the membranes were washed with 0.1% TBS-T. An HRP-detection system was used to visualize protein bands (Super Signal West Dura Extended Duration Substrate, 34076, Pierce Biotechnology, Thermo Scientific, Rockford, IL, USA). Quantification was done using the ChemiDoc™ MP (Bio-Rad Laboratories, Inc.) detection system. Protein band intensities were calculated using Image Lab software (ver. 5.1, Bio-Rad Laboratories, Inc.). All protein band intensity measurements were normalized to the amount of protein measured in the membrane after blotting.[23]

2.6 | Immunohistochemistry

Eight μm thick cross-sections were cut with a microtome at −20°C (CM1860 UV, Leica Microsystems GmbH, Nussloch, Germany) and mounted on microscope slides (Superfrost Plus, Thermo Fisher Scientific, Inc., Waltham, MA, USA). All cross-sections from the same participants were arranged on the same slides. The sections were air-dried and stored at −80°C until further analysis.

The αB-crystallin, HSP70, muscle fiber type, and tenascin-C analyses were conducted on separate cross-sections. All cross-sections were blocked in room temperature with 2% bovine serum albumin (BSA; A4503, Sigma Life Science, St Louis, MO, USA) in PBS-T (PBS, 524650, Calbiochem, EMD Biosciences; 0.05% Tween-20). The sections were incubated with the analysis-specific primary mouse monoclonal antibody, and an additional primary rabbit polyclonal dystrophin antibody (Abcam, Cambridge, UK) to stain the sarcolemma. Lastly, appropriate secondary antibodies (anti-rabbit Alexa Fluor 594 and anti-mouse Alexa Fluor 488, Life Technologies, Invitrogen, Rockford, IL, USA) were applied to the sections before incubation for 60 min in room temperature. All antibodies were diluted in the blocking buffer with a specific dilution ratio listed in Table 2. Between stages, the sections were washed 3 × 5 min (3 × 10 min for tenascin-C) in PBS-T. The sections were embedded in ProLong Gold Antifade Reagent with DAPI (P36935; Invitrogen Molecular Probes, Eugene, OR, USA) and left to cure overnight at room temperature.

For optimal staining on the sections, the primary antibodies (Table 2) required different BSA-blocking and incubation steps. Sections with HSP70 antibodies were blocked for 30 min and incubated for 2 h room temperature. αB-crystallin sections were blocked for 60 min (10% goat serum was added to the blocking buffer to reduce background noise) and incubated overnight at 4°C. Sections with myosin-heavy chain (MHC) type II antibodies (SC-71, developed by Schiaffino, S., obtained by DSHB, Iowa, IA, USA) were blocked for 30 min and incubated for 60 min at room temperature. Finally, the tenascin-C sections were fixed in 2% paraformaldehyde for 5 min

TABLE 2 Primary and secondary antibodies for Western blotting and immunohistochemistry, along with catalog number, concentrations, and applied buffer dilutions

Antibody	Cat#	Concentration	Dilution
Western blot:			
αB-crystallin[a]	ADI-SPA−222 F	1 mg/ml	1:4000
HSP70[a]	ADI-SPA−810 F	1 mg/ml	1:4000
Goat anti-Mouse IgG[b]	31430	0.8 mg/ml	1:30 000
Immunohistochemistry:			
αB-crystallin[a]	ADI-SPA−222 F	1 mg/ml	1:200
HSP70[a]	ADI-SPA−810 F	1 mg/ml	1:200
Tenascin-C[c]	MA5-16086	1 mg/ml	1:100
SC−71[d]			1:500
Dystrophin[e]	Ab15277		1:500
Secondary antibodies:			
Alexa Fluor 594[f]	A11005	2 mg/ml	1:200
Alexa Fluor 488[f]	A11001	2 mg/ml	1:200

[a]Enzo Life Sciences, Inc., Farmingdale, NY, USA.
[b]Thermo Fisher Scientific, Inc., Hanover Park, IL, USA.
[c]Thermo Scientific, Rockford, IL, USA.
[d]Schiaffino, S., obtained by DSHB, Iowa, IA, USA.
[e]Abcam, Cambridge, UK.
[f]Life Technologies, Invitrogen, Rockford, IL, USA.

followed by 10-min permeabilization in 0.2% triton X-100 in PBS, before blocked in 2% BSA with 5% goat serum in PBS-T, for 60 min. The sections were incubated in antibodies against tenascin-C (Thermo Scientific, Rockford, IL, USA) overnight at 4°C.

Images of the muscle sections were acquired using a high-resolution camera (DP72, Olympus Corp., Tokyo, Japan) mounted on a microscope (BX61, Olympus Corp., Japan) with a fluorescence light source (X-Cite 120PCQ; EXFO Photonic Solutions Inc., Mississauga, ON, Canada). Camera and software settings were fixed to be able to compare staining intensities between muscle sections within the same participants. Quantification of staining intensity was conducted using the Fiji distribution of ImageJ,[24] where the researcher was blinded for subject and time point.

For the HSP staining intensity analyses, a single image was acquired with a total of 213 ± 52 (range 73–322) fibers analyzed per cross-section. The fibers were related to their respective muscle fiber type (from a separate, sequential section) and average staining intensity per fiber type was calculated. Of these fibers, 70 ± 9% were type II. An increase in HSP staining intensity indicates bound proteins to cytoskeletal structures. Analysis of granular staining was conducted manually by eye, by determining the proportion of fibers with αB-crystallin granule stains in proportion to all the fibers. Here, multiple images with a total average of 880 ± 397 fibers (range 168–2176) were analyzed per section. Ruptured fibers and the outermost layer of the muscle section were excluded. For the tenascin-C analysis, one image was acquired per section, covering most of the muscle sample. An optimal signal-to-noise ratio for positive staining was set in the Fiji software and used for all images, and the percentage of the total cross-section with positive tenascin-C staining was calculated.

2.7 | Statistics

All the outcome variables were analyzed separately using a linear mixed model (MIXED procedure in SAS 9.4 Software, SAS Institute, Cary, NC, USA). The outcome variables were log-transformed, before analyzed as change scores. The effects were back-transformed to express percent or factor changes. Time and time × baseline were specified as fixed effects, with time treated as a nominal variable. The adjustment for baseline values was done to address regression to the mean effect. To deal with interdependency and unequal variances due to the repeated measurements design, the R matrix was specified with time and subject ID as blocks, and an *unstructured* covariance structure, using the REPEATED statement in SAS. Some models had convergence problems due to low sample size at the 48-h time point. In such cases, the 48-h time point was omitted from the models. Results are presented as point estimates with 95% confidence intervals (CI). Statistical significance level was set to $p < 0.05$. Standardized effect sizes (ES; effects divided by the SD of the baseline value) were utilized to indicate the magnitude of the effects. With the creatine kinase,

myoglobin, CMJ, and muscle soreness measures, baseline values for all players in Wiig et al[4] were used to provide a more robust standardization. Pearson correlation coefficient was applied to assess relationships between fold changes from pre-match, in the different outcome variables (Table 1), at the same time point (1 h, 24 h, 48 h, and 72 h) post-match.

3 | RESULTS

3.1 | Match Load, neuromuscular fatigue, and muscle soreness

The final scores from the three matches were 2–1, 2–1 and 6–3. The players' performed on average a total distance covered of 10114 ± 1002 (m), a high-speed running distance of 492 ± 195 (m), and a PlayerLoad^{TM} of 990 ± 147 (AU). One player was substituted after 68 min due to knee pain, all other players played a full 90-min match. The mean CMJ height at baseline was 42.5 ± 2.3 cm. Decreases in CMJ height of −8.4% (95% CI; −12.1 to −4.6, ES = −0.85, $p < 0.01$) at 1 h, −9.7% (CI; −12.6 to −6.7, ES = −0.98, $p < 0.01$) at 24 h, −4.7% (CI; −9.2 to 0.0, ES = −0.46, $p = 0.05$) at 48 h, and −2.6% (CI; −6.0 to 1.0, ES = −0.25, $p = 0.13$) at 72 h were observed (Figure 1). On a 5-point scale, muscle soreness increased by 0.68 units (CI; 0.01 to 1.3, ES = 1.00, $p = 0.047$) at 24 h and then decreased toward baseline level at 48 h (Figure 1).

3.2 | Muscle damage indicators in blood

At baseline, the mean creatine kinase value was 367 ± 225 U/L, increasing 2.31-fold (CI; 1.88 to 2.84, ES = 1.34, $p < 0.01$) at 1 h and peaking 2.67-fold (CI; 1.89 to 3.78, ES = 1.60, $p < 0.01$) at 24 h. At 72 h post-match, there was still a 1.77-fold increase (CI; 1.06 to 2.97, ES = 1.00, $p = 0.033$) compared to baseline (Figure 1). Myoglobin was 32 ± 10 (μg/L) at baseline and peaked at 1 h with a 9.95-fold increase (CI; 6.43 to 15.23, ES = 4.12, $p < 0.01$), returning to a 1.88-fold increase (CI; 1.49 to 2.38, ES = 1.06, $p < 0.01$) at 24 h compared to baseline. At 48 and 72 h, factor increases of 1.44 and 1.65 were observed, respectively, but with larger uncertainty (Figure 1).

3.3 | Western blot

In the cytosolic fraction, αB-crystallin decreased by a factor of 0.83 (CI; 0.75 to 0.92, ES = −0.73, $p < 0.01$; Figures 2 and 3) at 1 h and returned to baseline values at 24 h post-match. A secondary decrease in αB-crystallin was observed 48 h after the match, however with a large

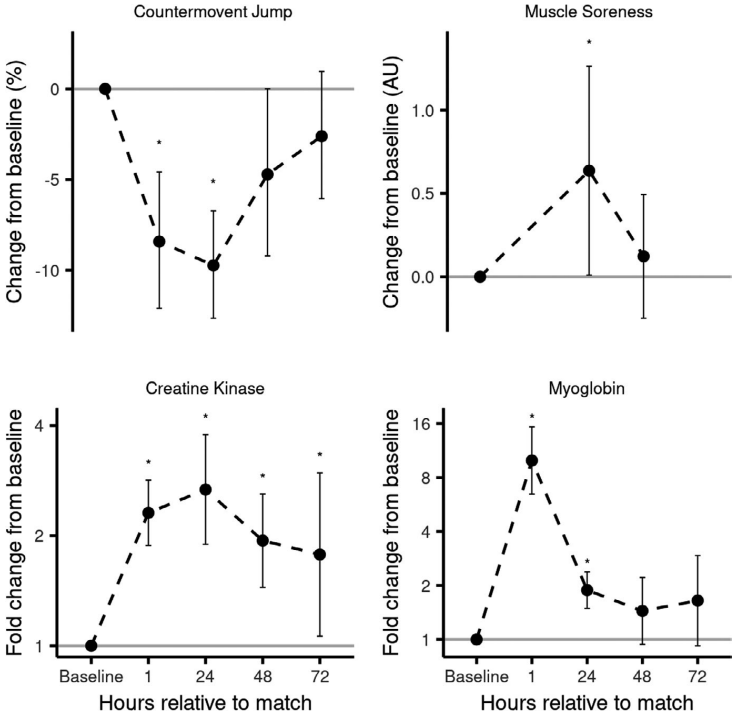

FIGURE 1 Change in countermovement jump, perceived muscle soreness, creatine kinase, and myoglobin from baseline in the period after the match. Uncertainty in the estimates is indicated by 95% CI. * different from baseline values ($p < 0.05$)

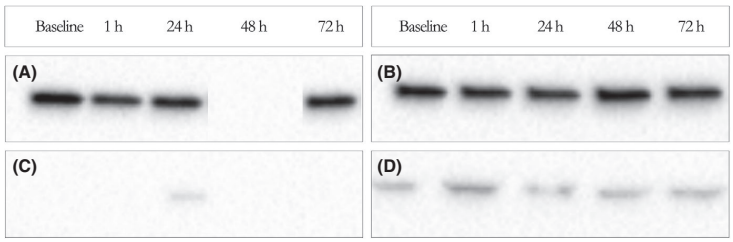

FIGURE 2 Western blot bands for αB-crystallin, showing cytosol levels (A) and cytoskeleton levels (C). HSP70 bands show cytosol levels (B) and cytoskeleton levels (D). The missing band in the 48h αB-crystallin lane (A) was due to this subject missing the 48 h biopsy

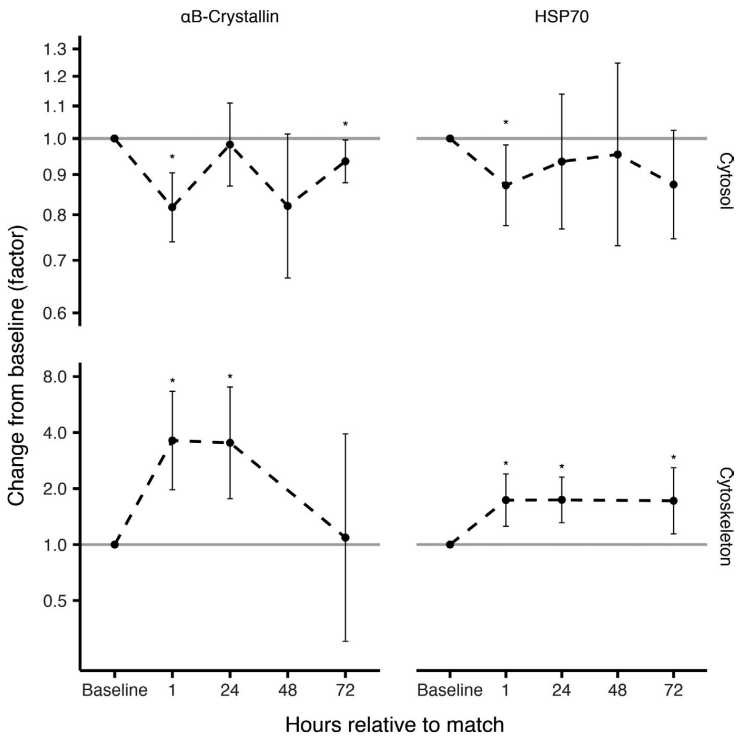

FIGURE 3 αB-crystallin and HSP70 measured by Western blotting as factor change from baseline, in the period after the match. Upper panes show the cytosol fraction, whereas the lower panes show the cytoskeletal fraction. Uncertainty in the estimates is indicated with 95% CI. * different from baseline values ($p < 0.05$)

uncertainty (CI; 0.65 to 1.10). In the cytoskeletal fraction, a 3.63-fold increase (CI; 1.98 to 6.66, ES = 4.94, $p < 0.01$) was observed 1 h post-match and αB-crystallin levels remained high in the cytoskeletal fraction until returning toward baseline level at 72 h.

Cytosolic levels of HSP70 decreased by a factor of 0.85 from baseline (CI; 0.76 to 0.95, ES = −0.78, $p = 0.010$) at 1 h post-match. HSP70 levels were still lower by a factor of 0.92–0.93 (CI; 0.73 to 1.18) at 24 and 48 h and 0.83 (CI; 0.68 to 1.02, ES = −0.89, $p = 0.072$) at 72 h post-match compared to baseline, but with greater uncertainty and therefore statistically non-significant. In the cytoskeletal fraction, HSP70 levels increased 1.78-fold (CI; 1.26 to 2.49, ES = 2.79, $p < 0.01$) at 1 h and remained approximately at the same levels until 72 h. The increase in the cytoskeletal fraction observed at 1 h corresponded to roughly 10% of the total cytosolic plus cytoskeletal levels at baseline.

3.4 | Immunohistochemistry

Analysis of muscle fiber types revealed that the players had a larger proportion of type II fibers (66%, CI, 60 to 71) compared to type I fibers. Staining intensity of both αB-crystallin and HSP70, in each fiber type, showed similar patterns with an increase from baseline at 24 h, a peak at 48 h and a reduction to approximately the 24 h levels at 72 h (Figure 4).

Specifically, αB-crystallin increased by 22% (CI; 7 to 39, ES = 0.82, $p < 0.01$) in the type I fibers and 27% (CI; 11 to 46, ES = 1.05, $p < 0.01$) in the type II fibers at 24 h and

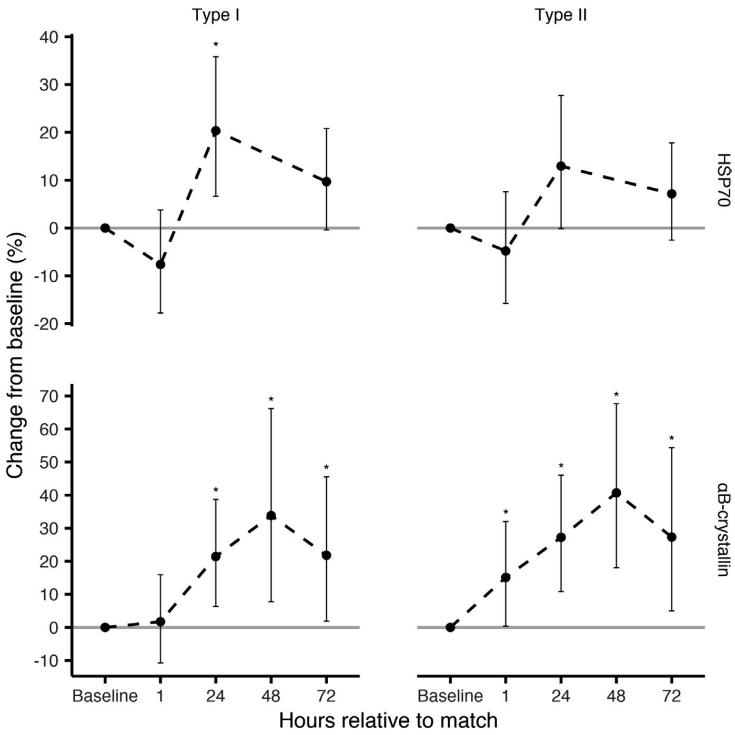

FIGURE 4 Percent change in staining intensity from baseline for αB-crystallin and HSP70, measured by immunohistochemistry, in the period after the match. Left panes show HSP response in type I muscle fibers, whereas right panes show HSP response in type II muscle fibers. Uncertainty in the estimates is indicated with 95% CI. * different from baseline values ($p < 0.05$)

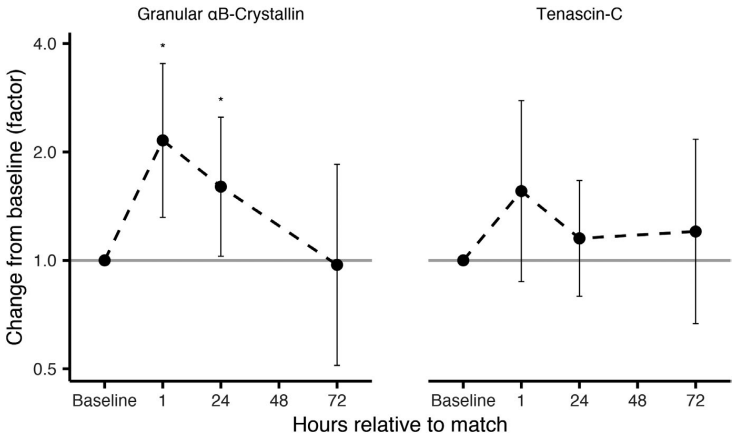

FIGURE 5 Fold change from baseline to specific time point post-match for granular staining of αB-crystallin and for tenascin-C. Uncertainty in the estimates is indicated with 95% CI. * different from baseline values ($p < 0.05$)

was still significantly elevated at 72 h. HSP70 showed a 20% increase (CI; 6 to 36, ES = 0.93, $p < 0.01$) in type I fibers and a 13% (CI; −0.1 to 27, ES = 0.76, $p = 0.052$) in the type II fibers at 24 h, however, at the other time points the confidence intervals also covered negative values (Figure 4). While αB-crystallin staining intensity peaked at 48 h (both fiber types), it is unknown whether HSP70 peaked at 24 h or 48 h due to the missing time point at 48 h.

Granular staining of αB-crystallin was observed in 1.0 ± 0.7% of the fibers at baseline. The proportion of fibers with granular staining increased at 1 h by a factor of 2.2 (CI; 1.3 to 3.6, ES = 1.21, $p < 0.01$) and 1.6 at 24 h (CI; 1.0 to 2.5, ES = 0.71, $p = 0.046$). At 72 h, the proportion of granular stained fibers returned to baseline level, although with large uncertainty (Figures 5 and 6). Granular staining was observed in both fiber types. At baseline, 0.93 ± 0.52% of the analyzed area showed immunoreactive

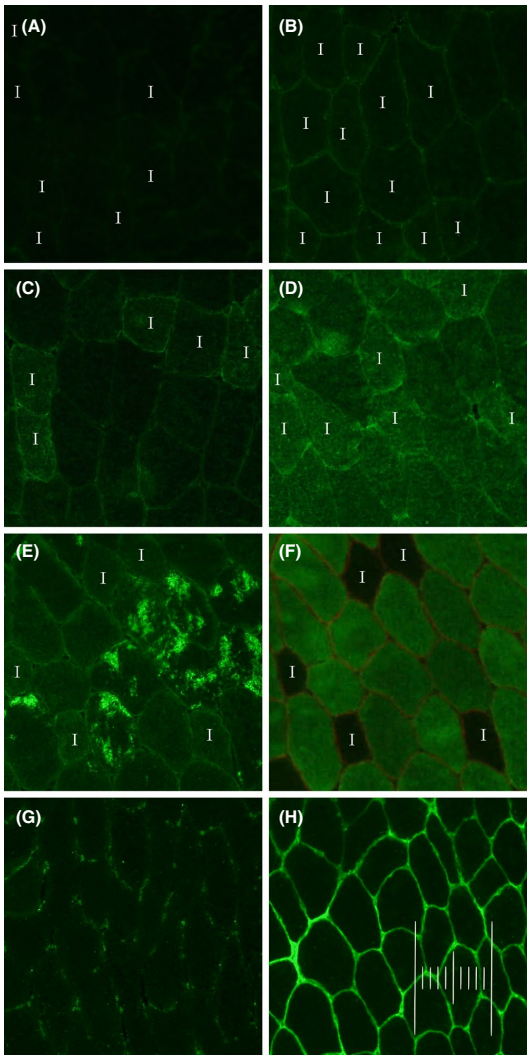

FIGURE 6 Cross-sections of muscle fibers after immunohistochemistry showing: HSP70 staining at baseline (A) and 24 h (B), αB-crystallin staining at baseline (C) and 24 h (D), muscle fibers with substantial granular staining of αB-crystallin at 1h (E) and a corresponding, adjacent cross-section differentiating muscle fiber type I (black) and fiber type II (F), and tenascin-C immunoreactive staining in a subject with substantial differences between baseline (G) and 72 h post-match (H). Fiber type I is labeled on pictures A–F, and a scalebar of 100 μm is printed on picture H

tenascin-C. The observed average stained area increased by factors of 1.56, 1.15, and 1.20 at 1 h, 24 h, and 72 h, respectively, with great uncertainty, but all effects were statistically non-significant ($p > 0.05$; Figures 5 and 6).

3.5 | Correlations

No statistically significant correlations were observed between change in any HSP measures and change in CMJ, CK, or myoglobin after the match. Change in staining intensity levels were correlated in type I and type II fibers in αB-crystallin ($r = 0.88$, $p < 0.01$) and HSP70 ($r = 0.96$, $p < 0.01$) at 1 h post-match. Furthermore, changes in cytoskeletal αB-crystallin levels were correlated with changes in αB-crystallin staining intensity in type I ($r = 0.74$, $p < 0.01$) and type II ($r = 0.74$, $p < 0.01$) fibers at 1 h post-match. For HSP70, however, correlation effects were trivial. Changes in creatine kinase and myoglobin were strongly correlated ($r = 0.92$–0.98, $p < 0.01$) across all time points.

4 | DISCUSSION

In the present study, muscle HSP stress response, blood markers for muscle damage, muscle soreness, and neuromuscular fatigue were assessed in semi-professional football players 1, 24, 48, and 72 h post-match. The main findings were that (1) HSP70 and αB-crystallin showed an immediate accumulation in cytoskeletal structures of muscle fibers indicated by an 1.8- and 3.6-fold increase in HSP70 and αB-crystallin in the cytoskeletal fraction at 1 h; (2) a 12%–27% increase in HSP70 and αB-crystallin staining intensity at 24 h and 72 h; and (3) the cellular response was accompanied by a decrease in CMJ height, an increase in muscle soreness, and increases in creatine kinase and myoglobin blood levels.

4.1 | HSP response to football match

The current findings suggest that a 90-min football match elicits a heat shock protein response in the muscle fibers immediately post-match and that it remains elevated 48–72 h after the match. Specifically, an increase in HSP70 and αB-crystallin were observed consistently in both the cytoskeletal fraction of the homogenate and on the muscle cross-sections, which suggests that the HSPs bind to damaged structural proteins.[12] A further indication of damaged structural proteins was the observation of granular staining of αB-crystallin. Such accumulation of HSPs is shown to be co-localized with myofibrillar disruptions.[12] Our results show that approximately 1% of the fibers had granular staining of αB-crystallin at baseline, which doubled to 2.2% (range: 0%–10%) at 1 h post-match.

The magnitude of the αB-crystallin response in the cytoskeletal fraction was similar to Frankenberg et al.[25] Where a 30-min eccentric step exercise was performed. However, such translocation of HSPs did not occur

following a repeated bout 8 weeks later, suggesting that the HSP response was due to unaccustomed eccentric exercise and that the muscle fibers were protected against muscle damage at the second bout. Such protection is also seen in trained subjects exposed to high-intensity running.[26] However, in our study, the HSP response was still evident despite that the football players were well trained and were used to playing weekly matches and therefore should be accustomed to the match load. This suggests that the physical load in football matches is substantial and that the intensity in parts of a match exceeds the stress tolerability in some muscle fibers. One explanation for the lack of protection from previous football matches could be that a much broader range of supra-threshold eccentric and concentric movements are executed in football compared to the more uniform movements that are seen in step exercises and running. Such movements would affect a larger range of substructures that may differ from situation to situation and match to match and may not allow for a near full protection. On the other hand, we observed a substantially smaller stress response and less reduction in muscle function after the football matches than has been observed after more extreme muscle-damaging exercise protocols.[14,27] Such protocols have caused HSP27 to increase 10–15 fold in the cytoskeletal fraction and the force-generating capacity in the quadriceps to be substantially decreased even one week post-exercise.

In both Western blot (cytoskeletal fraction) and immunohistochemistry analyses, HSP70 showed a weaker but longer lasting response than αB-crystallin. In line with other studies,[12] αB-crystallin recovered faster than HSP70 in the cytoskeletal fraction, although this was not evident on the immunohistochemical analyses. The different time patterns may reflect their different roles, as αB-crystallin is thought to be related to acute binding to damaged structural proteins to prevent protein aggregation,[28] whereas HSP70 is more related to refolding and controlled removal of damaged proteins.[15] It is well known that HSP70 also binds to newly synthesized proteins helping them to fold to the native state.[29] Hence, we cannot exclude that HSP70 is upregulated due to increased protein synthesis.

The observed external match load and the creatine kinase, myoglobin, and CMJ response post-match was comparable to other competitive matches.[1,30–32] Hence, it is reasonable to consider the HSP response as representative to a typical football match. The magnitude of the changes observed for creatine kinase, myoglobin, muscle soreness, CMJ height, and the HSPs suggests that a typical football match, for the average player, induce muscle damage which by Paulsen et al[8] could be categorized as mild. Despite our characterization of muscle damage apparently as mild, the observed recovery time for functional measures, such as CMJ, linear sprint performance, as well as perceptual measures such as muscle soreness, was still in the range of 72 h or more.[1,4] The concurrent HSPs response along with a reduction in CMJ performance indicates that muscle damage is one of the causes for the prolonged neuromuscular fatigue. It can also be speculated that the mild muscle damage could be the cause of the higher muscle injury rates observed when there is ≤3 compared to ≥6 days between matches. [33] In such cases, this mild form of muscle damage is pivotal and must be contended with in practice. Especially those players with the highest match loads and higher risk for more pronounced muscle damage and delayed recovery,[4] should be identified and prioritized in the recovery period between matches.

4.2 | Accumulation of HSPs in cytoskeletal structures

The rapid decrease of HSPs in the cytosolic fraction and the concurrent increase of HSPs in the cytoskeletal fraction suggest a translocation of the HSPs within the stressed myofibers. It indicates that the HSP changes from an unbound state in the cytosol, to bind to cytoskeletal structures.[13] Such translocation is usually seen in other studies after eccentric exercise[13,25,27] and muscle-damaging protocols,[12,14] but not after isometric contractions.[13,27] After muscle-damaging protocols we have shown that αB-crystallin accumulates in the Z-disc region, especially in sarcomeres with structural disruptions, and that granular αB-crystallin staining coincides with the sarcomere disruptions.[12] Hence, the observed increase in fibers with granular staining further supports the suggestion that myofibrillar disruptions occurred during matches and contributed to the long-lasting fatigue in this study.

4.3 | Muscle fiber type-specific stress response

Both the αB-crystallin and the HSP70 responses to football match play seem to be very similar in type I and type II muscle fiber types, also shown by their strong correlations at multiple time points (r = 0.88 to 0.99). This suggests that football match play stresses both muscle fiber types, a pattern that is also seen with glycogen depletion.[34] It could reflect the combination of high-intensity work which activates type II fibers, and the long duration of the match. Stressing both fiber types is in contrast to what is usually observed after high-load resistance exercise,[35] where the stress response occurred predominantly in type II fibers. On the other hand, low-load, concentric blood-flow-restricted exercise, is shown to mainly stress the type I

fibers (HSP70).[16] In more extreme muscle-damaging protocols with maximal eccentric muscle action, αB-crystallin and HSP27 responses are seen in both fiber types, while a HSP 70 response was mainly seen in type II fibers.[12] Consequently, the combination of prolonged metabolic stress and repeated short bursts of high-intensity accelerations and decelerations are likely to contribute to the HSP responses observed in both type I and type II fibers after the football match.

4.4 | Extracellular matrix and inter-individual differences

Exercise may also induce damage to extracellular structures in the muscles. Tenascin-c is a protein that contributes to the remodeling of collagen fibers in the extracellular matrix, and increases in tenascin-c have been observed in muscle-damaging exercise protocols along with exercise-induced, intra-cellular damage.[11] While an average increase in the immunoreactive area for tenascin-c was observed post-match in the current study, the inter-individual variability was large, which is illustrated by the wide confidence intervals (Figure 5). In studies showing a clear positive tenascin-c response, the subjects had been subjected to unaccustomed, high-force exercise.[11,20] The lack of a uniform response could therefore indicate that football matches have a fairly tolerably load for the extracellular matrix that most players are adapted to, but with individual deviations.

Individual differences in the response to the match were evident in several of the other measurements. For example, two of the players experienced a very high creatine kinase and myoglobin response after the match, combined with a secondary, additional increase at 72 h with creatine kinase values of 2400 and 3200 U/L and myoglobin values of 216 and 299 μg/L. Furthermore, one subject showed extensive granular staining at 1 h post-match (10% of muscle fibers). These individual cases suggest that some players experienced a more severe exercise-induced muscle damage. Hence, practitioners should have an individual focus, as some players, on some occasions, may have more extensive muscle damage which could require longer recovery time.

4.5 | Correlations between measurements

The match load in the current study caused significant creatine kinase, myoglobin, and CMJ responses comparable to typical football matches.[1] Furthermore, the HSP stress responses indicated that some ultrastructural muscle damage occurred. While in theory these measures may be linked, the responses in neither the blood markers nor the CMJ were correlated with the response of any of the reported HSP measures. One reason could be that creatine kinase and myoglobin also originate from muscles other than m. vastus lateralis. In fact, increase in creatine kinase and myoglobin levels post-match is positively related to the amount of high-speed running during the match,[4] an activity pattern where the hamstring musculature is highly activated. In contrast to our study, Paulsen et al[14] found a strong correlation between decreased force-generating capacity and the responses of HSP27 (which has similar a response to αB-crystallin) and HSP70 at 0.5 h after exercise. However, in that study the reduction in force-generating capacity ranged from −20 to nearly −80%, whereas in our study the range of CMJ response was only 4 to −15% at 1 h post-match, meaning that the signal-to-noise ratio in our study was too low to detect an association. In addition, it should be acknowledged that the sample size was rather small for correlations analyses, thus, the results should be interpreted with caution.

4.6 | Limitations

A limitation to the methodology is that the muscle tissue from a needle biopsy is a very small sample compared to the whole muscle, to the muscle group, and to the total number of muscle groups involved in running and jumping. Thus, indications of muscle damage based on the muscle samples could be under- or over-represented, and inferences are limited to the quadriceps muscle group only. Furthermore, the wide confidence intervals for the biopsy measure estimates could indicate somewhat low power, especially at the 48-h time point with only 7 subjects. The large uncertainty could be partly due to trying to infer from a small muscle sample to a large muscle group, as discussed above and be partly due to sampling and measurement error from the multi-step laboratory analyses. Also, real differences in responses between players due to individual characteristics (i.e., training status, age, and genetics), different playing positions, or differences in the external match load relative to their typical external match load could have contributed to the uncertainty. Due to the invasiveness of the study design, the matches were conducted two to three weeks after the end of the season, meaning that the training load before the match might have been different from a typical in-season match. The players were still training in this period, but they completed fewer sessions per week and no matches.

5 | CONCLUSIONS

In conclusion, football match play produced a muscular HSP stress response, increases in markers of muscle damage in blood, reduced CMJ performance, and increased perceived muscle soreness compatible with mild muscle damage. Such muscle damage could contribute to the prolonged recovery time after football matches. Specifically, the observation of HSPs accumulation in cytoskeletal structures and increased proportion of fibers with granular HSP staining indicates damage to myofibrillar proteins. Furthermore, football match play seems to stress both type I and type II muscle fiber types similarly. However, compared to experiments with muscle-damaging protocols or with protocols where the task is unaccustomed, the HSP stress response was moderate. Consequently, the players are adapted to football match play, but there are still loading patterns in match play that exceeds the tolerability threshold and results in muscle damage. Both the variation in individual match load and training status probably contribute to the large variation in neuromuscular fatigue and time needed for full recovery.

6 | PERSPECTIVES

In professional football, the high number of matches per year and few days between matches make it challenging for players to fully recover from the last match and be optimally prepared to the next match. Several studies have shown that it takes up to 3–4 days to recover neuromuscular fatigue and indirect blood markers for muscle damage,[1] and that match load have impact on recovery.[4] In agreement with this, our study suggests football matches lead to a HSP stress response in the muscle fibers compatible with mild muscle damage that lasts 48–72 h. This information is important input for coaches and practitioners who manage training load and recovery strategies to prevent non-contact injuries and optimize physical performance, but also for football associations to organize match schedules with sufficient recovery time between matches.

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CONFLICT OF INTEREST

None of the authors declare conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendices

Appendix A

NSD Study I

Approval letter for Study I from Norsk senter for forkningsdata (NSD)

Norsk samfunnsvitenskapelig datatjeneste AS
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Vår dato: 07.03.2014 Vår ref: 37923 / 3 / LT Deres dato: Deres ref:

TILBAKEMELDING PÅ MELDING OM BEHANDLING AV PERSONOPPLYSNINGER

Vi viser til melding om behandling av personopplysninger, mottatt 28.02.2014. Meldingen gjelder prosjektet:

37923	*Monitorering av belastning og tretthet i norsk toppfotball*
Behandlingsansvarlig	*Norges idrettshøgskole, ved institusjonens øverste leder*
Daglig ansvarlig	*Håvard Wiig*

Personvernombudet har vurdert prosjektet, og finner at behandlingen av personopplysninger vil være regulert av § 7-27 i personopplysningsforskriften. Personvernombudet tilrår at prosjektet gjennomføres.

Personvernombudets tilråding forutsetter at prosjektet gjennomføres i tråd med opplysningene gitt i meldeskjemaet, korrespondanse med ombudet, ombudets kommentarer samt personopplysningsloven og helseregisterloven med forskrifter. Behandlingen av personopplysninger kan settes i gang.

Det gjøres oppmerksom på at det skal gis ny melding dersom behandlingen endres i forhold til de opplysninger som ligger til grunn for personvernombudets vurdering. Endringsmeldinger gis via et eget skjema, http://www.nsd.uib.no/personvern/meldeplikt/skjema.html. Det skal også gis melding etter tre år dersom prosjektet fortsatt pågår. Meldinger skal skje skriftlig til ombudet.

Personvernombudet har lagt ut opplysninger om prosjektet i en offentlig database, http://pvo.nsd.no/prosjekt.

Personvernombudet vil ved prosjektets avslutning, 31.12.2017, rette en henvendelse angående status for behandlingen av personopplysninger.

Vennlig hilsen

Katrine Utaaker Segadal

Lis Tenold

Kontaktperson: Lis Tenold tlf: 55 58 33 77
Vedlegg: Prosjektvurdering

Personvernombudet for forskning

Prosjektvurdering - Kommentar

Prosjektnr: 37923

Prosjektet gjennomføres i samarbeid med Norsk Toppfotballsenter, Universitetet i Oslo, Institutt for informatikk, Universitetet i Tromsø, Institutt for informatikk. Norges idrettshøgskole er behandlingsansvarlig institusjon. Personvernombudet forutsetter at behandlings-/ansvarsfordelingen formelt er avklart mellom institusjonene. Vi anbefaler at det utarbeides en avtale som omfatter ansvarsfordeling, ansvarsstruktur, hvem som initierer prosjektet, bruk av data og eventuelt eierskap.

Det gis skriftlig informasjon og innhentes skriftlig samtykke for deltakelse. Personvernombudet finner informasjonsskrivet tilfredsstillende utformet i henhold til personopplysningslovens vilkår.

Det vil i prosjektet bli registrert sensitive personopplysninger om helseforhold, jf. personopplysningsloven § 2 nr. 8 c).

Datamaterialet anonymiseres ved prosjektslutt, 31.12.2017 ved at verken direkte eller indirekte personidentifiserbare opplysninger fremgår, verken hos Norges Idrettshøgskole, Universitetet i Tromsø, Institutt for informatikk eller Universitetet i Oslo, Institutt for informatikk. Anonymisering innebærer at direkte personidentifiserende opplysninger som navn/koblingsnøkkel slettes, og at indirekte personidentifiserende opplysninger (sammenstilling av bakgrunnsopplysninger som f.eks. yrke, alder, kjønn) fjernes eller grovkategoriseres slik at ingen enkeltpersoner kan gjenkjennes i materialet.

Appendix B

REK Study II

Application for Study II to Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst)

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Silje U. Lauvrak	22845520	19.11.2015	2015/1869 REK sør-øst D
			Deres dato:	Deres referanse:
			22.09.2015	

Vår referanse må oppgis ved alle henvendelser

Truls Raastad
Norges idrettshøgskole

2015/1869 Kampbelastning og påfølgende restitusjon i fotball

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk (REK sør-øst D) i møtet 28.10.2015. Vurderingen er gjort med hjemmel i helseforskningsloven § 10, jf. forskningsetikkloven § 4.

Forskningsansvarlig: Norges idrettshøgskole
Prosjektleder: Truls Raastad

Prosjektleders prosjektbeskrivelse
Det tar opptil 72-96 timer å restituere seg etter en fotballkamp, men ofte er det bare 72t til neste kamp, noe som øker skaderisikoen betydelig. Økt forståelse av kampbelastning, tretthet og restitusjon er derfor viktig for å kunne redusere skaderisikoen og restitusjonstiden og å øke prestasjonsevnen. Akselerometerteknologi gjør det nå mulig å måle den fysiske belastningen i detalj og assosiere den med restitusjonsprosessene. Nitti spillere spiller én ordinær fotballkamp med bærbare mikrosensorer (inkl. akselerometer, gyroskop, magnetometer, GPS, pulsmåler). Et longitudinelt design benyttes hvor det før, 1, 24, 48 og 72 timer etter kampen gjennomføres fysiske tester og subjektive rapporteringer for å overvåke restitusjonsprosessene og prestasjonsevnen. Blodprøver (n=48) og muskelbiopsier (n=12) tas for å undersøke i hvilken grad en kamp fører til ultra-strukturelle muskelødeleggelser, og hvordan musklenes glykogenlagre benyttes.

Vurdering
Formålet med prosjektet er å øke kunnskapen om den fysiske belastningen i en fotballkamp og den påfølgende restitusjonen. I protokollen beskrives formålet « å undersøke sammenhengen mellom kampbelastning (med fokus på akselerometerdata) og tretthet, restitusjon og prestasjon» Komiteen vurderer at prosjektet, slik det er presentert i søknad og protokoll, ikke vil gi ny kunnskap om helse og sykdom som sådan. Prosjektet faller derfor utenfor REKs mandat etter helseforskningsloven, som forutsetter at formålet med prosjektet er å skaffe til veie "ny kunnskap om helse og sykdom", se lovens § 2 og § 4 bokstav a).

Det kreves ikke godkjenning fra REK for å gjennomføre prosjektet. Det er institusjonens ansvar å sørge for at prosjektet gjennomføres på en forsvarlig måte med hensyn til for eksempel regler for taushetsplikt og personvern samt innhenting av stedlige godkjenninger.

Vedtak
Prosjektet faller utenfor helseforskningslovens virkeområde, jf. § 2 og § 4 bokstav a). Det kreves ikke godkjenning fra REK for å gjennomføre prosjektet.

Klageadgang
REKs vedtak kan påklages, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst D. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst D, sendes klagen videre

til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Vi ber om at alle henvendelser sendes inn på korrekt skjema via vår saksportal: http://helseforskning.etikkom.no. Dersom det ikke finnes passende skjema kan henvendelsen rettes på e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen

Finn Wisløff
Professor em. dr. med.
Leder

Silje U. Lauvrak
Rådgiver

Kopi til: turid.sjostedt@nih.no
Norges idrettshøgskole ved øverste administrative ledelse: postmottak@nih.no

Appendix C

NSD Study II

Approval letter for Study II from Norsk senter for forkningsdata (NSD)

Norges idrettshøgskole
e-postadresse
Att: Håvard Wiig
havard.wiig@nih.no

Vår dato: 21.09.18 *Vår ref: 60363/3/HJT/RH* *Deres dato:* *Deres ref:*

VURDERING AV BEHANDLING AV SÆRSKILTE KATEGORIER PERSONOPPLYSNINGER I «KAMPBELASTNING OG PÅFØLGENDE RESTITUSJON I FOTBALL»

NSD - Norsk senter for forskningsdata AS viser til meldeskjema innsendt 16.04.18. Meldingen gjelder behandling av personopplysninger til forskningsformål.

Prosjektet ble gjennomført høsten 2015, noe som innebærer at datamaterialet alt er samlet inn. Opprinnelige ble prosjektet meldt til REK, som vurderte at det falt utenfor helseforskningsloven. Videre ble det vurdert av en lokal etisk komité ved Norges idrettshøgskole (NIH). Grunnet svikt i interne rutiner ved NIH ble prosjektet ikke meldt til NSD slik det burde. Dette innebærer et brudd på meldeplikten i følge den tidligere personopplysningslovens § 31, jf. § 7-27. Prosjektet ble igangsatt uten vurdering fra NSD.

Dette innebærer at behandlingen av personopplysninger har foregått uten lovlig behandlingsgrunnlag siden 2015. Ved en gjennomgang av interne rutiner ved NIH ble avviket oppdaget.

Vi minner om den behandlingsansvarliges ansvar for internkontroll, jf. personvernforordningens artikkel 24 nr. I. Den behandlingsansvarlige må gjennomføre tiltak for å sikre og påvise at behandling av personopplysninger skjer i samsvar med forordningen. NSD gjør oppmerksom på at NIH er pliktig å dokumentere ethvert brudd på personopplysningssikkerheten, herunder de faktiske forhold, virkningene av det og hvilke tiltak som er truffet for å utbedre det. Dette skal gjøres uavhengig av om avviket meldes til Datatilsynet eller ikke.

Etter dialog mellom NSD og NIH ble prosjektet meldt til NSD 16.04.18, som her har foretatt en vurdering av hvorvidt innsamlingen av personopplysninger som har blitt gjennomført og den planlagte videre behandlingen er i samsvar med personvernlovgivningen.

Resultat av NSDs vurdering:

NSD vurderer at det har blitt behandlet særskilte kategorier personopplysninger (navn, telefonnummer, bilde- eller videoopptak, fysiske tester) og at opplysningene skal behandles frem til 31.12.19, og senere lagres avidentifisert til 2030.

NSDs vurdering er at behandlingen vil være i samsvar med personvernlovgivingen, og at lovlig grunnlag for behandlingen er samtykke.

Vår vurdering forutsetter at prosjektansvarlig behandler personopplysninger i tråd med:
- opplysninger gitt i meldeskjema og øvrig dokumentasjon
- dialog med NSD, og vår vurdering (se under)

NSD – Norsk senter for forskningsdata AS Harald Hårfagres gate 29 Tel: +47-55 58 21 17 nsd@nsd.no Org.nr. 985 321 884
NSD – Norwegian Centre for Research Data NO-5007 Bergen, NORWAY Faks: +47-55 58 96 50 www.nsd.no

- Norges idrettshøgskole sine retningslinjer for datasikkerhet, herunder regler om hvilke tekniske hjelpemidler det er tillatt å bruke
- Norges idrettshøgskole sine retningslinjer for bruk av databehandler

Nærmere begrunnelse for NSDs vurdering:

1. Beskrivelse av den planlagte behandlingen av personopplysninger

Det tar opptil 72-96 timer å restituere seg etter en fotballkamp, men ofte er det bare 72 timer til neste kamp, noe som øker skaderisikoen betydelig. Formålet med prosjektet har vært å øke forståelsen av kampbelastning, tretthet og restitusjon, som er viktig for å kunne redusere skaderisikoen og restitusjonstiden og å øke prestasjonsevnen.

Akselerometerteknologi gjør det mulig å måle den fysiske belastningen i detalj og assosiere den med restitusjonsprosessene. 81 spillere spilte én ordinær fotballkamp med bærbare mikrosensorer (inkl. akselerometer, gyroskop, magnetometer, GPS, pulsmåler). Før, 1, 24, 48 og 72 timer etter kampen ble det gjennomført fysiske tester, spørreskjema, blodprøver (n=60), og muskelbiopsier (n=12) for å overvåke restitusjonsprosessene og prestasjonsevnen.

Utvalget i prosjektet bestod av til sammen 81 mannlige fotballspillere i alderen 16-35 fra 6 ulike fotballag, spillende på 2.divisjonsnivå i Oslo/Akershus området. Rekruttering ble foretatt fra norsk 2.divisjon, herrer, hvor lagene er på et tilstrekkelig høyt prestasjonsnivå, og hvor treningsmengde og antall kamper i tilsvarer profesjonell fotball. Lagene som ble kontaktet holder til i Oslo/Akershus-regionen.

Daglig ansvarlig kontaktet trener for hvert lag. Deretter ble det holdt et informasjonsmøte for spillerne på lagene som var interessert i å være med. Spillerne fikk muntlig og skriftlig informasjon og det ble innhentet samtykke fra den enkelte spiller.

Datamaterialet har blitt samlet inn ved hjelp av papirbasert spørreskjema, fysiske tester og måleinstrumenter, samt en mobil-app. Materialet består av navn, telefonnummer, videoopptak, blodprøver, muskelbiopsi, puls og andre fysiske mål under fotballkamp.

All behandling av personopplysninger i prosjektet har vært, og er basert på utvalgets informerte samtykke.

I følge meldeskjema skal personopplysningene behandles frem til 31.12.19. I følge informasjonsskrivet lagres de deretter avidentifisert i 15 år, til 2030, før de anonymiseres.

2. Personvernprinsipper

NSDs vurdering er at behandlingen følger personvernprinsippene, ved at personopplysninger;

- skal behandles på en lovlig, rettferdig og åpen måte med hensyn til den registrerte (se punkt 3 og 4)
- skal samles inn for spesifikke, uttrykkelig angitte og berettigede formål og der personopplysningene ikke viderebehandles på en måte som er uforenelig med formålet (se punkt 1 og 3)
- vil være adekvate, relevante og begrenset til det som er nødvendig for formålet de behandles for (se punkt 8)
- skal lagres slik måte at det ikke er mulig å identifisere de registrerte lengre enn det som er nødvendig for formålet (se punkt 6 og 8)

3. Lovlig grunnlag for å behandle særskilte kategorier personopplysninger

Selv om forsker, av grunner nevnt innledningsvis, ikke oppfylte meldeplikten i forkant av prosjektstart, vurderer NSD at den planlagte behandlingen av personopplysninger er lovlig fordi det ble innhentet uttrykkelig samtykke fra de registrerte.

Samtykket innhentes skriftlig ved at den registrerte signerer på samtykkeskjema i papirform.

4. De registrertes rettigheter

NSD vurderer at den registrerte har krav på å benytte seg av følgende rettigheter: informasjon, innsyn, retting og sletting av personopplysninger.

NSD finner at informasjonsskrivet datert oktober 2015 vil gi de registrerte adekvat informasjon om hva behandlingen innebærer og om hvilke rettigheter de har.

Utvalget bestod av personer over 16 år. NSD er enig i at 16-åringer kan samtykke selv til deltakelse i prosjektet. I følge meldeskjema var det én 15-åring med, og det ble i dette tilfellet innhentet samtykke fra hans foreldre.

Vi minner om at hvis en registrert tar kontakt om sine rettigheter, har Norges idrettshøgskole plikt til å svare innen en måned. Prosjektansvarlig har da ansvar for å etterinformere den registrerte om nye tilleggsrettigheter i følge GDPR: dataportabilitet, begrensning, og protest. Vi forutsetter at prosjektansvarlig informerer institusjonen så fort som mulig og at NIH har rutiner for hvordan henvendelser fra registrerte skal følges opp.

5. Nødvendige tillatelser

I følge meldeskjema er deler av datamaterialet (blodprøver og muskelbiopsi) lagret i forskningsbiobank, vurdert av REK. Ansvarlig for biobanken er dr. Truls Raastad. Prøver og avidentifiserte opplysninger deles med Universitetet i Odense for analyseformål.

NSD ber om at vi får ettersendt REKs vurdering av forskningsbiobanken.

I avsnittet om biobank i informasjonsskrivet opplyses det om at prøvene skal lagres til 2028.

6. Informasjonssikkerhet

I følge meldingen behandles personopplysningene ved hjelp av mobile lagringsenheter, notater/papir, server i virksomhetens nettverk og på datamaskin i nettverkssystem tilknyttet internett tilhørende virksomheten, samt i biofryser (biobank).

Koblingsnøkkelen oppbevares i safe som bare daglig ansvarlig har direkte tilgang til. Andre medarbeidere har bare tilgang via daglig ansvarlig.

Tilgang til datamaskin er beskyttet av brukernavn og passord. Alle data er kun registrert med forsøksnummer.

Data som samles inn via mobil-appen overføres via internett og lagres på server hos databehandler. Deltakere som ønsker å få utlevert sine data kan få dette på kryptert epost.

NSD forutsetter at personopplysningene behandles i tråd med personvernforordningens krav og institusjonens retningslinjer for informasjonssikkerhet.

7. Databehandler

I følge meldingen er iAD SFI ved Universitetet i Tromsø benyttet som databehandler. Det foreligger databehandlingsavtale mellom NIH og databehandler.

8. Varighet

Ifølge meldeskjema skal personopplysninger behandles frem til 31.12.19, for deretter å lagres avidentifisert til 2030. Opplysninger som kan knyttes til en enkeltperson skal da slettes/anonymiseres.

Norges idrettshøgskole må kunne dokumentere at datamaterialet er anonymisert.

Anonymisering innebærer å bearbeide datamaterialet slik at ingen enkeltpersoner kan bli identifisert. Det gjøres ved å:

- Slette navn, fødselsnummer/andre ID-nummer, adresse, telefonnummer, epostadresse, IP-adresse og andre nettidentifikatorer
- Slette biologisk materiale (som blodprøver, vevsprøver)
- Slette eller grovkategorisere alder, bosted, arbeidssted, institusjon, diagnose, lokaliseringsdata og andre bakgrunnsopplysninger
- Slette eller sladde videopptak og lydopptak

Meld fra om endringer

Dersom behandlingen av personopplysninger endrer seg, kan det være nødvendig å melde dette til NSD via Min side. På våre nettsider informerer vi om hvilke endringer som må meldes. Vent på svar før endringen gjennomføres.

Informasjon om behandlingen publiseres på Min side, Meldingsarkivet og nettsider

Alle relevante saksopplysninger og dokumenter er tilgjengelig:

- via Min side for forskere, veiledere og studenter
- via Meldingsarkivet for ansatte med internkontrolloppgaver ved Norges idrettshøgskole.

NSD tar kontakt om status for behandling av personopplysninger

Etter avtale med Norges idrettshøgskole vil NSD følge opp behandlingen av personopplysninger ved planlagt avslutning.

Vi sender da en skriftlig henvendelse til prosjektansvarlig og ber om skriftlig svar på status for behandling av personopplysninger.

Se våre nettsider eller ta kontakt ved spørsmål. Vi ønsker lykke til med behandlingen av personopplysninger.

Med vennlig hilsen

Marianne Høgetveit Myhren
seksjonsleder

Håkon Jørgen Tranvåg
rådgiver

Lovhenvisninger
NSDs vurdering er at den planlagte behandlingen av personopplysninger:

- er regulert av personopplysningsloven, jf. § 2.
- oppfyller prinsippene i personvernforordningen om:
  - lovlighet, rettferdighet og åpenhet jf. art. 5.1 a)
  - formålsbegrensning jf. art. 5.1 b)
  - dataminimering jf. art. 5.1 c)
  - lagringsbegrensning jf. art. 5.1 e).
- kan finne sted med hjemmel i personvernforordningen art. 6.1 a), art. 9.2 a), jf. personopplysningsloven § 9, jf. § 10
- gjennomføres på en måte som ivaretar de registrertes rettigheter personvernforordningen **art. 11-22**

NSD legger til grunn at institusjonen også sørger for at behandlingen gjennomføres i samsvar med personvernforordningen:

- art. 5.1 d) og art. 5.1. f) og art. 32 om sikkerhet
- art. 26-29 ved felles behandlingsansvar med andre institusjoner eller bruk av databehandler