TITLE:

Muscle fibre typology as a novel risk factor for hamstring strain injuries in professional football (soccer): a prospective cohort study

RUNNING HEAD:

Muscle fibre typology and hamstring injury

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Eline Lievens received a grant from Research Foundation-Flanders (FWO 1104020N), during the conduct of the study; Kim Van Vossel, Freek Van de Casteele, Evi Wezenbeek, Dieter Deprez, Bram De Winne, Steve McNally, Wolter De Graaf, James B Murdoch, Jan G Bourgois, Erik Witvrouw and Wim Derave declare that they have no conflict of interest.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the local ethical committee (Ghent University Hospital, Ghent, Belgium; B670201837890).

Consent to participate

All subjects gave their written informed consent to take part in the study.

Consent for publication

Not Applicable

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability

Not Applicable

Author's contributions

Eline Lievens, Wim Derave, Erik Witvrouw participated in the design of the study and drafted the manuscript. Kim Van Vossel, Freek Van de Casteele, Evi Wezenbeek contributed to the statistical analysis. Dieter Deprez, Stijn Matthys, Bram De Winne, Steve McNally, Wolter De Graaf, James B Murdoch and Jan G Bourgois participated in data collection. All authors contributed to data interpretation and reviewed the final version.

ABSTRACT:

Background

Hamstring strain injuries (HSI) are prevalent in team sports and occur frequently in the latter phase of matches. In the search for interindividual factors that determine muscle fatigue and possibly injury risk, muscle fibre typology is a likely candidate. It was the aim of the study to determine whether muscle fibre typology is a risk factor for HSI.

Methods

A prospective cohort study was conducted over 3 seasons in professional football players competing in the Belgian Jupiler Pro League (n=118) and in the English Premier League (n=47), and a total of 27 hamstring strain injuries were sustained during this period. The muscle fibre typology was non-invasively estimated using proton magnetic resonance spectroscopy, and was characterized as a fast, slow, or intermediate typology based on the carnosine concentration in the soleus. A multivariate cox model was used to identify risk factors for HSI.

Results

Football players exhibit a wide variety of muscle typologies (slow typology=44.9%; intermediate typology=39.8%, fast typology=15.3%). In the combined cohort, players with a fast typology displayed a 5.3-fold (95% CI 1.92-14.8, P=0.001) higher risk than slow typology players to sustain an index HSI. This was also independently observed in both leagues separately as respectively a 6.7-fold (95% CI 1.3-34.1; P=0.023) and a 5.1-fold (95% CI 1.2-20.4; P=0.023) higher chance was found in fast typology players than slow typology players of the Jupiler Pro League and the Premier League cohort.

Conclusion

We discovered muscle fibre typology as a novel and potent risk factor for HSI in team sports.

Key points

- We identify muscle fibre typology as an important risk factor for HSI in football, with fast typology players displaying a 5.3-fold higher risk than slow typology players for experiencing a new HSI.

- In the continous analysis, a hazard ratio of 1.83 was found (95% CI 1.25-2.66; P=0.002), which means that when the Z-score increases by one unit (indicating a faster typology), an athlete will have 83% more chance to get a HSI.
- The muscle fibre typology was non-invasively estimated by proton magnetic resonance spectroscopy, accommodating its use in elite sport.

1. INTRODUCTION

Hamstring strain injuries (HSI) are the most prevalent non-contact injuries in many team sports, including football in which they are responsible for 12% of all injuries[1]. Given the high incidence and the recurrence rate (16%, characterized by an even longer rehabilitation), these injuries have a major impact on sport performance, as well as on sports medical costs[1–3]. A number of risk factors for HSI have been characterised, including increasing age, previous hamstring injury, lower eccentric hamstring strength and shorter fascicle length[4,5]. Despite decades of intensive scientific attention, the HSI incidence is not declining[3], which could indicate that the nature of the problem is insufficiently understood or that the compliance in current prevention strategies is insufficient. On the other hand, the fact that the high-intensity running distance during football games has markedly increased over the years [6] but has not led to increased HSI incidence, could indicate at least a partial success in knowledge translation.

HSI are more frequently sustained in the latter phase of matches, highlighting muscle fatigue as a potential contributing factor to injury susceptibility[1,7]. While evidence for an association between fatigue and hamstring injury is principally epidemiological, there are a number of fatigue-induced alterations that could theoretically increase susceptibility to injury[8]: altered muscle activation and neuromuscular coordination[9,10], altered pelvic position[10], reduction in proprioceptive ability[11,12], decreased lower limb muscle stiffness[13] and reduced eccentric hamstring strength[5,10,14–16]. In the search for interindividual factors that determine increased muscle fatigue -and thus possibly HSI risk- the muscle fibre type composition is a likely candidate since fast-twitch muscle fibres are less resistant to fatigue than slow-twitch fibres[17]. It can therefore be hypothesized that players with a predominant fast typology (FT) exhibit more fatigue during matches and are as such at increased risk for HSI.

Direct evidence of dominant fast-twitch (or slow-twitch) fibre composition being a risk factor for muscle strain injuries in vivo is currently lacking. This is probably because the gold standard to measure the muscle fibre type composition, i.e. a histological evaluation of a muscle needle biopsy, has significant limitations and is not feasible in a large cohort of professional (football) players. Our research group has developed a novel non-invasive approach to estimate muscle fibre typology through the measurement of muscle carnosine, a characteristic metabolite of fast-twitch fibres, by proton magnetic resonance spectroscopy (^{1}H-MRS)[18]. More precisely, the muscle typology was estimated in the soleus, as this muscle was found to be the most reliable during test-retest[19], exhibited the lowest biological variability[20] and allowed to compare the values of these football players to a large reference population of healthy, non-specifically trained individuals. Moreover, due to the presence of an across-muscle phenotype, implying that the fibre type

proportion of one muscle is indicative of the proportion in the body as a whole, the muscle fibre typology of the hamstrings will be related to the typology in the soleus[21,22]. This approach therefore enables investigation as to whether muscle fibre typology is a risk factor for muscle strain injury.

This study aimed to explore the muscle fibre typology of professional football players (research question 1). As professional football requires both endurance capacity and sprint ability[6], it was hypothesised that football teams provide a heterogeneous group of muscle typologies, that would allow us to assess the muscle fibre typology as a risk factor for HSI incidence (research question 2). The 1-3 year follow-up was done in 165 adult professional football players. Moreover, since this study included 2 different cohorts: the Jupiler Pro League in Belgium and the Premier League in the UK, we also examined if the findings were preserved when analysing the two professional leagues individually and separately. We hypothesised that a faster typology would be linked to a higher risk for HSI.

2. METHODS

2.1. Study design

This study was conducted over 3 consecutive seasons (2015-2018) in four teams of the Belgian Jupiler Pro League and one team of the Premier League. Throughout this period, the muscle fibre typology of 165 unique adult professional football players (Jupiler Pro League: n=118; Premier League: n=47) was non-invasively determined. The Jupiler Pro League players were used to determine the muscle typology of football players relative to a reference population (Research question 1; Fig. 1). For the HSI risk assessment players of both leagues were included. Moreover, players were excluded if they were goal keepers, if they had a HSI in the six months before the screening (to avoid investigating re-injuries), if they left the team before the minimum follow-up of 1 year was reached, if the injury follow-up data was either not provided (2 players of the Premier League) or was not done with enough accuracy (occurred in one Jupiler Pro League team). After applying these exclusion criteria, sixty-one Jupiler Pro League players (age: 26.7 ± 4.79 y; weight: 76.2 ± 7.15 kg; height: 181 ± 7.18 cm; median (interquartile range) duration of exposure: 471 days (360 days)) and 34 Premier League players (age: 22.2 ± 1.76 y; weight: 72.4 ± 6.68 kg; height: 181 ± 6.38 cm; median (interquartile range) duration of exposure: 510 days (411 days)) were included in the HSI risk study.

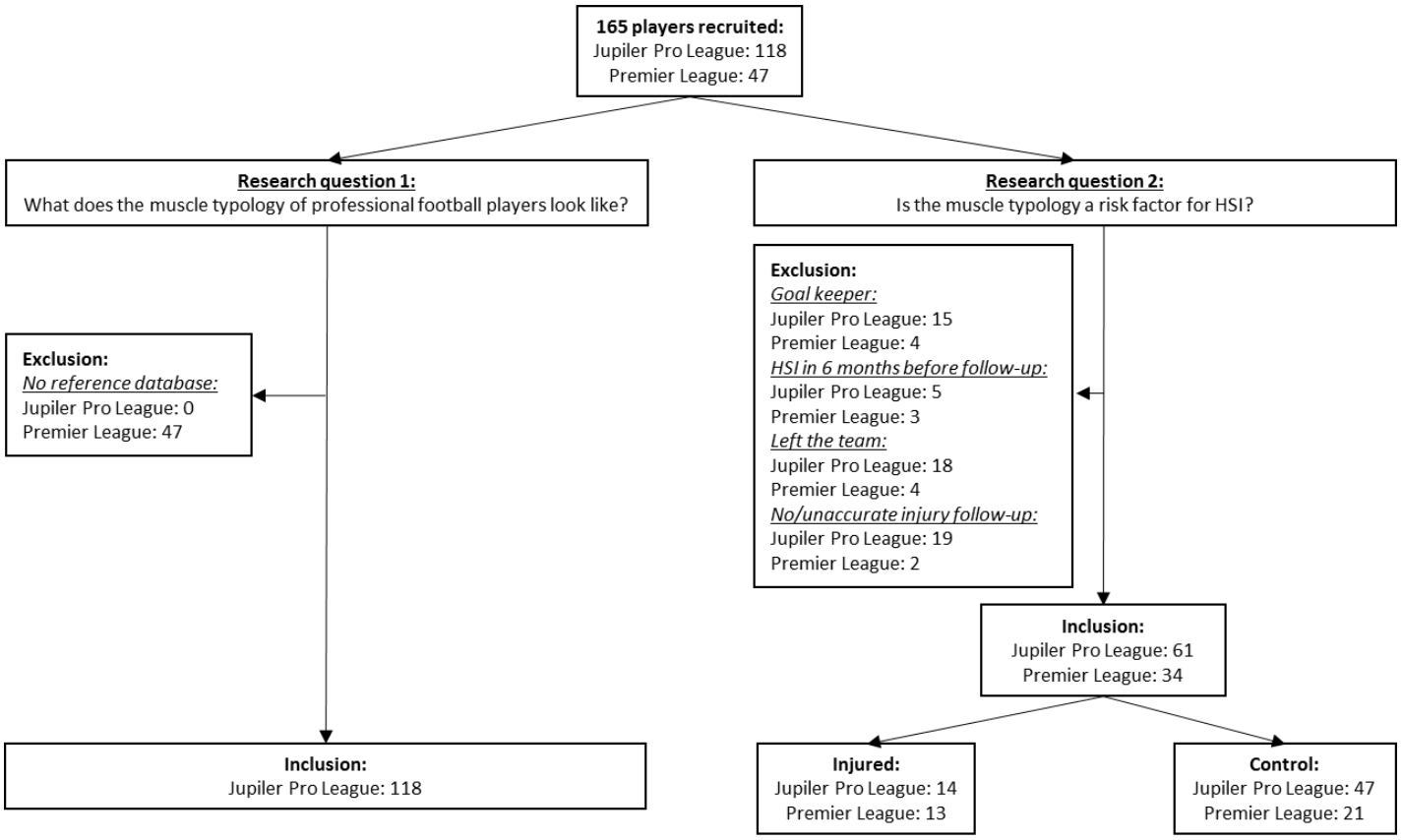

Fig. 1 Flowchart. HSI, hamstring strain injury

2.2. Muscle fibre typology assessment

Muscle carnosine content was measured by ^{1}H-MRS in the right soleus muscle on a 3T whole body MRI scanner (Jupiler Pro League: Trio, Siemens; Premier League: Vantage Titan, Canon), based on the size of the carnosine peak at 8 ppm. Subjects lay in a supine position and the lower leg was fixed in a knee coil (Jupiler Pro League) or a flexible wrap coil (Premier League). A single-voxel point-resolved spectroscopy (PRESS) sequence with the following parameters was used for the carnosine scan: repetition time (TR) of 2000 ms, echo time (TE) of 30 ms (Jupiler Pro League) / 32 ms (Premier League), 128 excitations, 1024 data points, spectral bandwidth of 1200 Hz (Jupiler Pro League) / 2604 Hz (Premier League), acquisition time of 4.27 min, and a voxel size of 40 mm x 12 mm x 30 mm. A representative spectrum is displayed in Fig. 2.

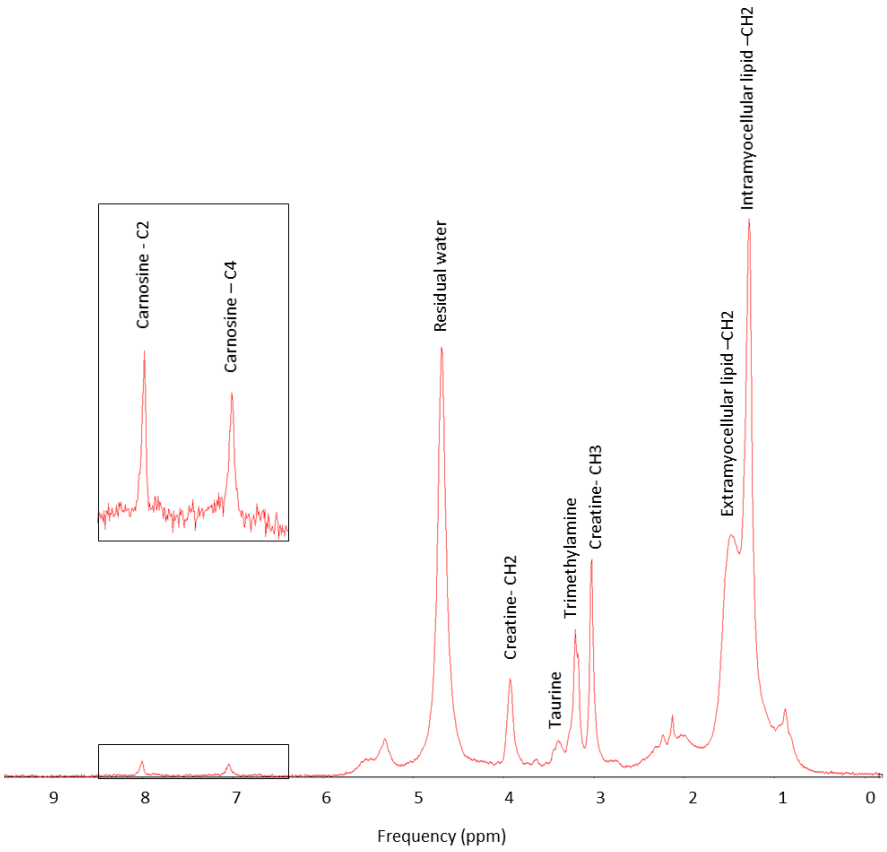

Fig. 2 Representative proton magnetic resonance spectrum of the soleus

The absolute carnosine content (mM) in the Jupiler Pro League was calculated as described by Baguet et al.[18], using the phantom replacement technique. We previously used this technique (albeit on different test groups) to determine the methodological and biological variability of the muscle carnosine content in the soleus. The methodological test-retest variation within the same day was found to be 4.3% (n=9) [19] and the biological variation over 15 weeks, 5-6 months, 1, 2 and 3 years was found to be respectively 9.8% (n=7)[20], 6.5% (n=17), 6.9% (n=11), 10.5% (n=7) and 8.8% (n=6) (unpublished observations). These values suggest that an increase in the time interval does not change the variation of the measurement.

In the Premier League, the carnosine content (mM) was calculated using unsuppressed water (eight excitations) as an internal reference. The formula used for this internal reference technique is:

Carnosine concentration (mM) = [(Signal$_{carnosine}$/Signal$_{water}$)][(Correction factor T1$_{water}$)/(Correction factor T1$_{carnosine}$)]*[(Correction factor T2$_{water}$)/(Correction factor T2$_{carnosine}$)]*(Concentration of water in the muscle)*number of protons in water.*

Carnosine concentration (mM) = [(Signal$_{carnosine}$/Signal$_{water}$)][(1-exp(-2000/1340))/(1-exp(-2000/1488))*[(exp(-32/28·7))/(exp(-32/152))]*(0.7*55000)*2.*

The relaxation correction factors for carnosine were earlier described by Baguet et al.[23] and the correction factors for water by MacMillan et al.[24]. The concentration of water in a muscle was deduced from the molar concentration of water (55000 mM) and the approximate water content of skeletal muscle tissue (0.7 L/kg wet weight of tissue)[25]. The methodological test-rest variation using this technique was found to be 4.0% (unpublished data; n=3). Moreover, a cross-vendor comparison was performed on a Siemens and a Canon 3T scanner (n=7), in which a good correlation (R^2=0.862) was found between soleus carnosine concentrations at both devices[26].

In the Jupiler Pro League, the carnosine concentrations were converted into Z-scores relative to an age- and sex-matched control population of 98 active, healthy non-specifically trained individuals. This allowed us to compare the Z-scores of the football players to the general population. A negative Z-score indicates a slower muscle fibre typology when compared to the general population, while a positive Z-score indicates a faster muscle fibre typology. In addition, the Z-scores of the football players were compared to Z-scores of elite athletes in typical sprint-type sports (n=15; 100-200m running, BMX and track-sprint cycling disciplines) and endurance-type sports (n=61; road cycling, mountain bike, triathlon, and 1500m to marathon running), which were scanned on the same device.

In the Premier League, the carnosine concentrations were converted into a Z-score relative to all male football players scanned on the same device (n=65), as we did not have an independent reference database on a Canon system. For both cohorts, football players were assigned into 3 groups based on their Z-score: a slow typology (ST) group with a Z-score≤-0.5, an intermediate typology (IT) group with a Z-score between -0.5 and 0.5, and a fast typology (FT) group, with a Z-score≥0.5.

2.3. Hamstring strain injury assessment

Prior to the scan, personal data (age, height, weight, position) were provided by the medical staff. During the follow-up period, the type and duration of all injuries were documented. The exposure for each player was determined by

subtracting the injured period (days) from the overall follow-up period (days). The endpoint of the follow-up period was either the end of season 2017- 2018, the day that the player left the team, or that he sustained a HSI. A HSI was defined as acute pain in the posterior thigh that occurred during training or match, which resulted in the inability to participate in the next training session or match, and was later diagnosed by the club medical staff. In two of the Jupiler Pro League clubs, a hamstring strain was diagnosed clinically with the support of echography. Depending on the size and the location of the injury, an MRI was consulted for a more in depth diagnosis. In one Jupiler Pro League team and the Premier League team, more than 95% of the hamstring stains were MRI-confirmed.

2.4. Statistics

2.4.1. Muscle fibre typology assessment:

A one way ANOVA was used to determine if the muscle fibre typology Z-scores were different between playing positions in both leagues separately. The following assumptions for a one way ANOVA were determined: (1) Normality of the data was assessed in the 4 different position groups both visually and through the Shapiro-Wilk normality test. The latter was significant in defenders of the Premier League. However visual assessment showed a positive skewness, which was not interpreted as problematic. (2) Homogeneity of the data was assessed by Levene's tests, which were non-significant. (3) Samples were independent in the Jupiler Pro League and the Premier League.

2.4.2. Hamstring strain injury risk assessment:

A multivariable Cox model was used to determine the relation between the muscle fibre typology (Z-score based on carnosine content) and the sustained HSI. Following assumptions of the multivariable Cox model were determined: (1) Proportional hazard assumption was assessed by a log minus log analysis and was satisfied both for the Jupiler Pro League, Premier League and the combined cohort, (2) the linearity of continuous covariates was assessed using a Martingale residuals scatterplot, (3) samples were found to be independent in the Jupiler Pro League and the Premier League, in the combined cohort, league was added as a covariate and (4) censoring was found to be non-informative.

We investigated if correction was needed for anthropometric variables, position, age and league. First a directed acyclic graph was compiled, showing that correction might be needed for age and league. Next, univariate Cox model analyses were preformed allowing reductions of the number of variables, since variables were only included for further analysis if $P<0.2$. Second, multivariate Cox model analyses were performed to identify significant contributors to the development of HSI. To calculate the receiver operating characteristic curves (ROC), an equal time of exposure for all

players is a prerequisite and therefore only the follow-up from the first year was used. The area under the curve (AUC) and the specificity and sensitivity of the model were determined.

3. RESULTS

3.1. Muscle fibre typology assessment:

Players from the Jupiler Pro League exhibit a much wider variety of muscle typologies than elite athletes from individual sport disciplines. In contrast to the sprint and endurance sports, football players were more evenly distributed in all 3 main categories (ST=44.9%; IT=39.8%, FT=15.3%). These muscle fibre typology distributions did not differ between playing positions in the Jupiler Pro League or in the Premier League (Fig. 3; P=0.432 and P=0.798, respectively).

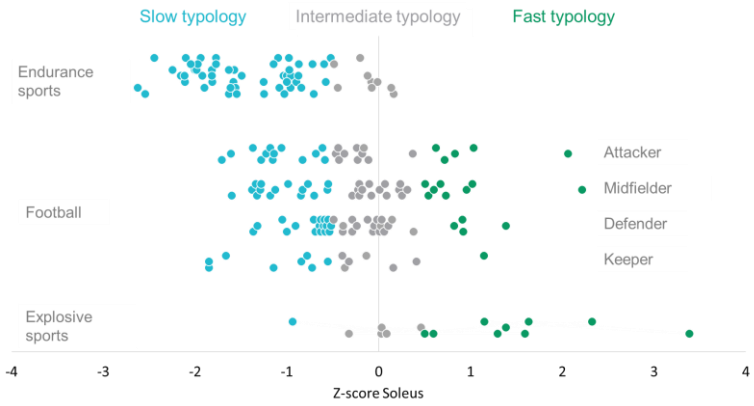

Fig. 3 Muscle fibre typology of Jupiler Pro League players compared to athletes from individual sport disciplines. Values are expressed as Z-scores. Football is further subdivided into the different field positions.

3.2. Hamstring strain injury risk assessment:

In the combined cohort, a total of 27 HSI were reported during the follow-up period. When correcting for age and league, the muscle fibre typology Z-score showed a hazard ratio (HR) of 1.83 (95% CI 1.25-2.66; P=0.002; Table 1). This means that when the Z-score increases by one unit, an athlete will have 83% more chance to get a HSI. In the Jupiler Pro League (14 HSI) a HR of 2.05 (95% CI 1.15-3.66; P=0.015) was found, when correcting for age. Moreover, these findings were mirrored in the Premier League (13 HSI), in which a HR of 1.81 was found (95% CI 1.07-3·05; P=0.026).

When dividing the players into ST, IT and FT categories (as shown in Fig. 4), the HSI incidence in the combined cohort, the Jupiler Pro League and the Premier League was found to be respectively 5.32-fold (95% CI 1.92-14.8, P=0.001), 6.66-fold (95% CI 1.30-34.1; P=0.023) and 5.05-fold (95% CI 1.24-20.4; P=0.023) higher in the FT than in the ST players.

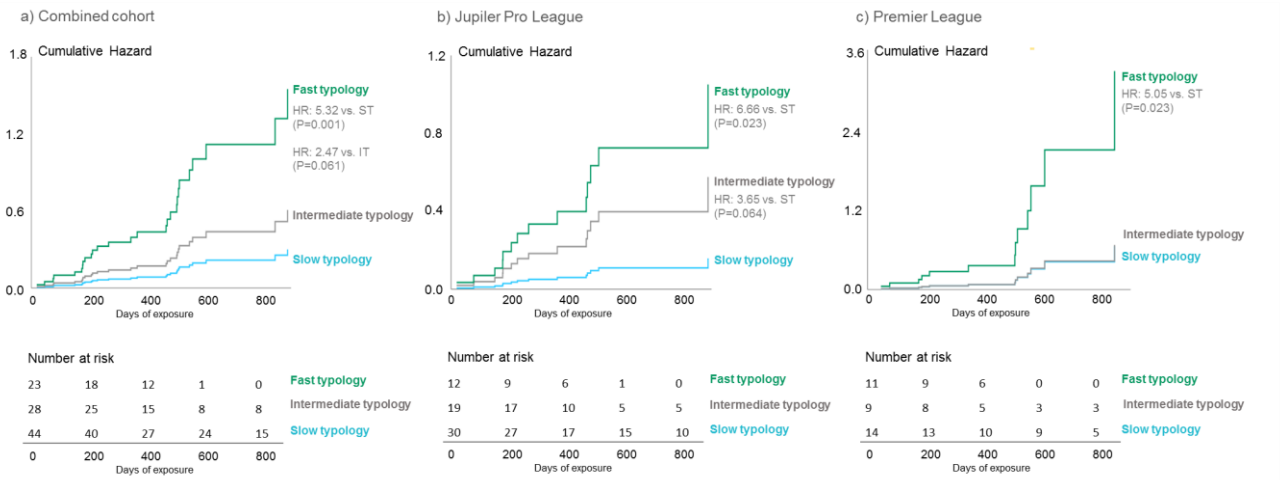

Fig. 4 Cumulative hazard curves and number at risk in a) Combined cohort, b) Jupiler Pro League and c) Premier League. HR, hazard ratio

ROC curve analysis of the variables muscle fibre typology, age and league (Table 1) revealed an AUC of 0.669, 0.752 and 0.804 in respectively the combined cohort, Jupiler Pro League and the Premier League, indicating poor to good combined sensitivity and specificity.

TABLE 1

Multivariable Cox model with hazard ratio's (HR) and 95% confidence intervals (CI) and ROC analysis with area under the curve (AUC), 95% CI, sensitivity, and specificity

		Multivariable Cox model (1-3 year)				ROC analysis (1 year)			
	Variable	**HR**	**95% CI**	**P value**	**HSI (n)**	**AUC**	**95% CI**	**Sensitivity/specificity**	**HSI (n)**
Combined cohort	Z-score soleus	1.83	1.25 to 2.66	**0.002**	27	0.669	0.501 to 0.838	0.714/0.593	14
	Age	1.07	0.948 to 1.200	0.283					
	League	1.80	0.688 to 4.687	0.232					
Jupiler Pro League	Z-score soleus	2.05	1.15 to 3.66	**0.015**	14	0.752	0.531 to 0.974	0.750/0.717	8
	Age	1.13	0.996 to 1.29	0.057					
Premier League	Z-score soleus	1.81	1.07 to 3.05	**0.026**	13	0.804	0.636 to 0.971	0.833/0.607	6
	Age	0.552	0.310 to 0.981	**0.043**					

The bolded P values indicate a statistically significant factor in the analysis

4. DISCUSSION

The present study demonstrated heterogeneous muscle typologies in a large cohort of football players. Some players have muscles that resemble those of endurance athletes, some are intermediate and other players resemble explosive sprint-type athletes. The clear heterogeneity in the present study allowed us to investigate muscle fibre typology as a potential risk factor for HSI.

The main finding of the present study is that the muscle fibre typology is strongly related to the risk for HSI. Football players with a FT display a 5.3-fold higher risk than ST players in sustaining an index HSI, and this was independently confirmed in 2 different leagues (Jupiler Pro League and Premier League). Muscle fibre typology seems to be an important risk factor, as these values are higher than the previously proposed risk factors such as higher age (HR=1.1) and hamstring injury history (HR=3.5)[27]. Moreover the sensitivity and specificity, described by the area under the ROC curve (AUC=0.67-0.80), are comparable with eccentric knee flexor torque and fascicle length (AUC=0.65; relative risk (RR)=4.4 and AUC=0.71; RR=4.1, respectively)[5].

It is generally acknowledged that a large portion of the biopsy-based muscle fibre typology is genetically determined[28]. A previous twin study, using the same non-invasive estimate of muscle fibre typology, likewise revealed a 85% heritability (95% CI 0.71–0.91) for the soleus carnosine content[29]. Whether or not this implies that the increased risk for HSI in FT players is genetically determined and therefore inevitable, depends on the potential causal mechanism of the relationship. We propose two explanations that would render the muscle fibre typology either a non-modifiable or a modifiable risk factor.

First, the higher vulnerability of FT players may be related to structural differences between fast- and slow-twitch muscle fibres[30]. Fast-twitch fibres contain narrower Z-disks, smaller isoforms of nebulin and myomesion and less elastic isoforms of the protein titin, which results in a lower sarcomeric integrity when compared to slow-twitch fibres. Moreover, dystrophin, which connects the cytoskeleton to the sarcolemma, has a twofold lower content in fast-twitch fibres compared to slow-twitch fibres. Also, the frequency of sarcomere branching, providing a pathway for lateral and longitudinal active force transmission, is 2.8-fold lower in fast-twitch than slow-twitch fibres[31]. Additionally, fast-twitch fibres can produce a higher power when compared to slow-twitch fibres, which results in an even larger imbalance between the higher load and the lower load capacity. In vitro studies show that the above described factors make the fast-twitch fibres inherently more vulnerable to strain-induced damage[30].

A second potential reason for the higher HSI risk in FT players is based on the link between fatigue and HSI. In a previous study from our lab, a priori identified FT subjects showed a higher fatigue accumulation during three Wingate tests and took 15 times as long to recover from this high-intensity exercise when compared to the ST group[32]. When generalising these data to football players, FT players might accumulate fatigue during their games, possibly resulting in a higher injury susceptibility. More specifically, it is suspected that hamstring injuries are mostly sustained during the terminal swing phase of high-intensity running, in which the hamstrings have to work eccentrically to control the knee extension force. If hamstring-related fatigue during these eccentric actions is predominantly present in FT players, their hamstrings might no longer be able to produce the high forces needed to control the knee extensions, possibly resulting in overlengthening and tears[33,34].

It remains to be determined to what extent these potential mechanisms (structural weakness and/or fatigue) contribute to our findings and whether they would work synergistically. In contrast to structural weakness, however, the higher fatigability might be partly modifiable by individualising training and recovery cycles. While most team players nowadays follow a one-size-fits-all training regimen, FT players may be disadvantaged and could instead benefit from individualisation strategies for their training-recovery cycles. We believe that individualisation based on player position (keeper/defender/midfielder/forward) will not meet these demands, as muscle typologies were somewhat equally distributed over all player positions in our study (see Fig. 3). Therefore non-invasive identification of muscle fibre typology groups, could be considered for more appropriate individualisation of training prescription and injury prevention strategies.

We acknowledge that the muscle fibre typology was non-invasively estimated in the soleus and not in the hamstrings, which can be seen as a limitation. However, due to the presence of an across-muscle phenotype, we believe that the muscle fibre typology of the hamstrings is related to the Z-score measured in the soleus muscle[21,22]. Moreover, the two different MRI systems used to measure the carnosine content in the soleus, introduced differences in the acquisition and in the reference population between sites. The relative low number of HSI in the Jupiler Pro League (n=14) and the Premier League (n=13) introduced the possibility of sparse data bias[35] and resulted in a low fragility index[36], therefore results should be interpreted with caution. However, the different study locations enabled to study larger groups and test the reproducibility of the findings, which increases the power of our findings.

5. CONCLUSION

Muscle fibre typology has been revealed as a novel risk factor for HSI in team sports, with FT players displaying a 5.3-fold higher risk than ST players in experiencing a new HSI. This risk may be partly modifiable when fatigue is involved in this relationship. Future research should use a multifactorial design in order to highlight the interrelatedness and importance of all established risk factors, in an attempt to lower future strains.

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