The Utility of a High-intensity Exercise Protocol to Prospectively Assess ACL Injury Risk

Authors

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Key words

- biomechanics
- anterior cruciate ligament
- isokinetic dynamometry

Abstract

▼ This study investigated the utility of a 5-min high-intensity exercise protocol (SAFT⁵) to include in prospective cohort studies investigating ACL injury risk. 15 active females were tested on 2 occasions during which their non-dominant leg was analysed before SAFT⁵ (PRE), immediately after (POST0), 15 min after (POST15), and 30 min after (POST30). On the first occasion, testing included 5 maximum isokinetic contractions for eccentric and concentric hamstring and concentric quadriceps and on the second occasion, 3 trials of 2 landing tasks (i.e., single-leg hop and drop vertical jump) were conducted. Results

showed a reduced eccentric hamstring peak torque at POST0, POST15 and POST30 ($p < 0.05$) and a reduced functional HQ ratio (Hecc/Qcon) at POST15 and POST30 ($p < 0.05$). Additionally, a more extended knee angle at POST30 ($p < 0.05$) and increased knee internal rotation angle at POST0 and POST15 ($p < 0.05$) were found in a single-leg hop. SAFT⁵ altered landing strategies associated with increased ACL injury risk and similar to observations from match simulations. Our findings therefore support the utility of a high-intensity exercise protocol such as SAFT⁵ to strengthen injury screening tests and to include in prospective cohort studies where time constraints apply.

Introduction

▼ Risk factors of ACL injuries can only be defined with the highest level of evidence when prospectively assessed [39]. Muscular and biomechanical ACL injury risk factors have been studied extensively as they are modifiable through training [41]. Muscular risk factors include reduced eccentric hamstring peak torque (H_{ecc}) and reduced hamstring/quadriceps ratio (H/Q) [45]. Reduced hamstring strength is believed to permit increased anterior tibial translation and in turn increase ACL strain [2]. Biomechanical risk factors include increased peak knee abduction moment and peak vertical ground reaction force ($VGRF_{peak}$) [21]. Reduced knee flexion at initial contact (IC) during landing has been associated with increased anterior tibial translation [6,7], which causes increased ACL loading [6,8,9]. Additionally, increased knee internal rotation has been associated with increased ACL loading [9,10]. It is important to note that fatigue (i.e., loss of maximum or potential performance) alters these factors which may further increase injury risk [11,12]. Additionally, evidence shows

that most injuries occur at the end of a soccer match [12,13]. These findings suggest that fatigue plays a crucial role on muscular and biomechanical risk factors in the mechanism of ACL injury [19]. To date, however, no prospective studies have included exercise-induced fatigue in their screening protocols, most likely due to the conflict between screening a large cohort and the time-consuming nature of inducing fatigue. Fatigue has been induced in a number of ways, some of which are more related to dynamic activities than others. Previous research has suggested, however, that including functional movements as part of the protocol is key to revealing specific match-play-induced deficits which can increase ACL injury risk [14,15]. As such the effects of a shuttle run revealed changes in transverse plane kinematics in sidestep cutting as increased external rotation of hip, knee and ankle angle at IC and increased knee internal rotation angle during stance [40]. In another study, a treadmill-based soccer match simulation reduced H/Q [16]. Finally, a soccer-specific match simulation (SAFT⁹⁰) which included multidirectional movements, high accelerations and decel-

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erations, and which was shown to be a valid simulation of match play [26], caused significant reductions in H_{ecc} and in H/Q [36,43,44]. Fatigue induced by soccer match play (90 min) has been suggested to be a combination of both central (altered motor commands from the motor cortex) and peripheral fatigue (metabolite accumulation, limitations in energy supply, reduced blood flow and neuromuscular mechanisms) [37,50]. Additionally it has been suggested that during soccer, players experience fatigue in several different ways: (1) disturbed muscle ion homeostasis during temporary fatigue after short bursts of high-intensity exercises, (2) lowered muscle temperature (e.g., at the beginning of the second half) and (3) through muscle glycogen and dehydration as experienced towards the end of a game [32]. Whilst full-length match simulations would be considered most ideal for simulating the effects of match play, the development and evaluation of short-term protocols is needed to include match-play-induced fatigue assessment within prospective studies. It is important to acknowledge the influence of intensity, duration and type of contraction on the mechanisms of fatigue [3]. As far as we are aware however, no previous study has directly compared neuromuscular responses between short-term protocols and full-length match simulations. Nevertheless, previous findings [27] found similar biomechanical alterations in response to a short-duration high-intensity protocol and a longer duration protocol (30 min). 2 studies involving short-term protocols, the first including vertical jumps followed by 30-m sprint, and the second including series of athletic exercises (countermovement jump (CMJ), step up/down, squat and shuttle run), resulted in decreased knee flexion angles at IC and increased knee abduction moments in sidestep cutting [10] and stop-jump tasks [8]. Finally, a short-term protocol based on continuous drills (step up/down and plyometric bounding) caused increased knee abduction angles/moments and increased knee internal rotation angles in a drop vertical jump (DVJ) [31]. It is important to note that all these protocols induced fatigue until maximum exhaustion which is not representative of match play, and inappropriate for the inclusion in prospective studies as this implies differences in duration or amount of repetitions.

Few if any studies have focused on short-term protocols that simulate match play of dynamic sports (i.e., sport which involves high accelerations and decelerations and typically involves interactions with an object (ball, racket, etc.)). Therefore, the aim of this study was to investigate how a short-term high-intensity exercise protocol (SAFT⁵), based on a long-duration match-play simulation (SAFT⁹⁰) [26], affects muscular and biomechanical markers of ACL injury in recreationally active females. It was hypothesized that SAFT⁵ would result in changes in markers of ACL injury risk.

Methods

Participants

15 females (age: 22 ± 3 years, height: 1.68 ± 0.07 m, mass: 70.8 ± 8.9 kg) were tested following a sample-size estimation from a functional fatigue protocol to observe a 5° difference in knee flexion angle at 80% statistical power and $\alpha=0.05$ [11,31]. All participants met the inclusion criteria: (1) female, (2) recreationally active (i.e., 3 sessions per week) in dynamic sports (hockey, netball, etc.), (3) did not suffer from an ACL injury and (4) did not suffer from any other lower limb injury within the last 6 months before data collection. Ethical approval was

granted by the university ethics committee and all participants provided informed consent according to IJSM ethical standards [18].

Design

This was a cross-sectional study with a repeated measures design. Initially participants were familiarized with the protocol and assessment methods, height and weight were recorded, and maximum jump height ($\text{JumpHeight}_{\max}$) was defined. This was followed by 2 testing sessions which were separated by at least 3 days. Both sessions included a dynamic warm-up, the SAFT⁵ protocol, a pre-test (PRE), a test immediately following SAFT⁵ (POST0), after 15 min of passive rest (POST15) and after 30 min of passive rest (POST30). These moments in time were selected in order to define the prolonged effect of SAFT⁵. This information is required for the inclusion of SAFT⁵ in a prospective study protocol which could consist of measurements before and after SAFT⁵. In addition, this information would be useful for the design of activity-rest cycles to minimize the potential for fatigue-related injury. Different parameters were assessed during each session and between participants the order of sessions was randomly assigned. Participants were instructed to avoid strenuous exercises 48 h prior to testing.

Exercise protocol

SAFT⁵ is based on the first 5 min of SAFT⁹⁰ [26,36]. The distance of the SAFT⁹⁰ protocol was modified to 15 m in order to make the SAFT⁵ course feasible in our laboratory (► Fig. 1 and ► Table 1). The intensity of SAFT⁵ was increased by adding high-intensity exercises, based on previous studies [11,31,51] and pilot work [9,54]. Pilot work aimed at defining the activity profile of the protocol by investigating the implementation of functional high-intensity movements. Several variations of the protocol based on different high-intensity exercises, amount of repetitions and order of exercises were explored by monitoring HR and RPE during the protocol which represented the intensity. The final protocol was selected in accordance with the following criteria: (1)

Fig. 1 The SAFT⁵ course. The participant received anticipatory instructions about the task and the speed for 5 min. The dotted line at the start of the course represents either upwards and backwards (“up jog”) or side-ways running (“side”) around the second cone. The solid line represents forward running and sidestep cutting around the middle pole. The speed of the task was either “jog” or “stride”. Stride represented a speed between a jog and sprint. Once the participant arrived back at the first pole a second instruction was given. This instruction was either: (1) “sprint” or “jog”, which meant that the participant jogged or sprinted; (2) “agility ladder”, which meant that the participant performed the agility drill of running forwards with one foot per square and performing a final sprint once finished with the agility ladder drill; (3) “CMJ” or “scissors”, which meant that the participant performed 10 CMJs (1 maximum jump on a jump mat and 9 jumps at 80% of $\text{JumpHeight}_{\max}$) or 10 scissors at the black square that is represented on the fig.

HR and RPE presented a similar overall pattern as during SAFT⁹⁰ and actual game play [24,36], and (2) practical and personal observations of the researchers. As such it was decided to include the following 3 exercises: a CMJ at 80% of their JumpHeight_{max}, an agility ladder drill (one foot per square) and a 'jump scissors' task (jumping from unilateral lunge with left leg forward and hands placed on the hips, to unilateral lunge with right leg forward) (► Table 1). During the protocol, white tape was placed onto a wall which represented 80% JumpHeight_{max}, participants had to touch the white tape during every jump and received verbal feedback if they didn't reach the tape.

Table 1 Activity profile of SAFT⁵ compared with the activity profile of 5 min of SAFT⁹⁰ with the order, speed and duration of each task represented.

No	Activity SAFT ⁵	Activity SAFT ⁹⁰	Speed (m/s)	Time (s)
0	10 × scissors			
1	stand	stand	0	4
2	jog	jog	2.86	10
3	CMJ (1 × CMJ _{max} + 9 × 80% JumpHeight _{max})	walk	1.39	17
4	stride	stride	4.17	7
5	agility ladder drill	walk	1.39	17
6	jog	jog	2.86	10
7	sprint	sprint	5.58	6
8	stand	stand	0	4
9	jog	jog	2.86	10
10	10 × Scissors	walk	1.39	17
11	jog	jog	2.86	10
12	CMJ (1 × CMJ _{max} + 9 × 80% JumpHeight _{max})	walk	1.39	17
13	stride	stride	4.17	7
14	agility ladder drill	walk	1.39	17
15	stand	stand	0	4
16	jog	jog	2.86	10
17	10 × Scissors	walk	1.39	17
18	jog	jog	2.86	10
19	jog	jog	2.86	10
20	jog	jog	2.86	10
21	CMJ (1 × CMJ _{max} + 9 × 80% JumpHeight _{max})	walk	1.39	17
22	jog	jog	2.86	10
23	agility ladder drill	walk	1.39	17
24	stride	stride	4.17	7
25	stand	stand	0	4
26	10 × scissors	walk	1.39	17
27	jog	jog	2.86	10
28	sprint	walk	1.39	17

Table 2 Timing and order of measurements (one maximum counter movement jump (CMJ)), heart rate (HR), rate of perceived exertion (RPE), kinematics and kinetics of a single leg hop (SLH) and drop vertical jump (DVJ) of the non-dominant leg and maximum voluntary contractions of concentric and eccentric hamstring strength and concentric quadriceps (IKD)) during the 2 testing sessions.

	PRE	SAFT 5							POST 0			POST 15		POST 30	
Time (min)	-15	-10	-5						0	5	10	15	20	25	30
1. CMJ															
2. HR															
3. RPE															
4. SLH&DVJ															
4. IKD															
Test 1&2															
Test 1															
Test 2															

Data collection

At one test session maximum voluntary contractions (MVCs) of concentric hamstring strength (H_{con}), concentric quadriceps strength (Q_{con}) and H_{ecc} were assessed by an isokinetic dynamometer (IKD, Biodex System 3, Shirley, NY). The non-dominant leg (i.e., non-preferred leg to kick a ball with) was tested as it has been identified as the most vulnerable to ACL injury in females [6,38]. Concentric MVCs consisted of repeated knee flexion and extension contractions within 90° RoM at 120°/s. During eccentric MVCs, participants resisted against the passive external knee extension moment of the IKD over 90° RoM and at 120°/s [35,43]. Participants were verbally encouraged and 5 repetitions were measured for each task with one min rest between different contractions. The order of assessment was randomly assigned.

At the other test session, participants wore tight-fitting clothes and measurements consisted of 3D motion and force analysis of DVJ and single-leg hop (SLH). For the DVJ, participants were instructed to drop off a 30-cm high box (feet 20cm apart), and land with each foot on separate force platforms, immediately rebounding for a maximum vertical jump. For the SLH, participants were instructed to stand on the non-dominant leg and hop forward to cover a distance of 75% of body height [33] in order to use a standardised distance adjusted to personal dimensions. 3 successful trials of each task were recorded with trials excluded if the participant lost balance less than 2 s after landing.

10 optoelectronic cameras sampling at 250 Hz (OQUS 3, Qualysis AB, Gothenburg, Sweden) were used to collect 3D motion data. Spherical reflective markers were attached to lower limb and trunk according to the LJMU kinematic model [49], which has established reliability [28]. One static and 4 functional motion trials were recorded to define functional hip and knee joint axes, after which anatomy-defining markers were removed. GRF were collected simultaneously from 2 force platforms at 1500 Hz (Kistler, Winterthur, Switzerland).

Additional measurements for both sessions, recorded every 5 min throughout the sessions and during SAFT⁵, included JumpHeight_{max} with a jump mat (Probotics, Inc., Huntsville, AL) in order to assess fatigue as a reduction of performance, heart rate (HR) (Polar heart rate system, Electro, Finland) and rate of perceived exertion (RPE) (20-point Borg scale). Timing and order of each measurement is represented in ► Table 2.

Data analysis

Kinematic and kinetic data were calculated within Visual 3D (C-Motion, Germantown, MD). Marker trajectories and forces

Fig. 2 Maximum jump height pre (–10 min to 0 min), during (0 min to 5 min) and post (5 min to 35 min) SAFT⁵. Vertical lines represent beginning (time –5) and end (time 0) of SAFT⁵. * denotes statistically significant difference ($p < 0.05$).

Fig. 3 Functional HQ ratio ($H_{eccentric}/Q_{concentric}$) and hamstring_{eccentric} peak torque pre, during and post (5 min, 20 min and 35 min) SAFT⁵. * denotes statistically significant difference ($p < 0.05$).

were filtered through a Butterworth and a critically damped low-pass filter with 20-Hz cut-off frequencies and normalized to 101 time nodes. IC and take-off were defined as the instant when GRF exceeded or reached below 10 N [10]. The stance phase of an SLH was defined as one second after IC. Only the first landing of the DVJ was used for analysis [1]. Euler rotations (X-Y-Z) were used for joint angle calculations and knee moments were obtained by inverse dynamics [31] and are reported as external moments. IKD data were gravity-corrected and analysed with a custom Matlab (MathWorks, Inc., Natick, MA, USA) program. Peak torques were calculated from a polynomial fit of data points that met the criteria within a 10% tolerance (velocity: $120^\circ/\text{s}$, RoM at least 70° for H_{con} and Q_{con} , and RoM at least 50° for H_{ecc}). Finally, the functional HQ ratio (H_{ecc}/Q_{con}) which was previously presented as a valid representation of muscle-specific exertion induced by football match play [12] and conventional

HQ ratio (H_{con}/Q_{con}) were calculated. Dependent variables of interest were $\text{JumpHeight}_{\max}$, HR, RPE, H_{ecc}/Q_{con} , H_{con}/Q_{con} , H_{con} , H_{ecc} and Q_{con} , knee flexion angle and transverse plane knee angle at IC, peak knee abduction moment and $\text{VGRF}_{\text{peak}}$.

Statistical treatment

Data are presented as means $\pm$ standard deviation. One-way repeated measures ANOVAs were used to assess meaningful variations across time for each variable. Percentage differences of jump height, H_{ecc} , H_{con} , Q_{con} , H_{ecc}/Q_{con} and H_{con}/Q_{con} were calculated and HR was presented as a percentage of the estimated maximum HR ($\text{HR}_{\max} = 220 - \text{age}$). If statistical significance was found, pairwise comparisons were applied with Bonferroni corrections to reduce the risk of type-1 errors. Reliability of the 2 testing sessions was confirmed by (1) low typical error and limits of agreement for RPE (Pre: 1, [–2; –1]; During: 1 [–1; 1]; Post: 1 [–1; 0]) and HR (Pre: 5 beats/min [–5; 3]; During: 2 beats/min [1; 4]; Post: 3 beats/min [–5; –1]) and (2) uniform errors based on observed homoscedasticity. All statistical analyses were performed in SPSS (Version 21.0, Chicago, IL) ($\alpha = 0.05$).

Results

There was a significant effect of SAFT⁵ on HR ($F_{3.99, 47.88} = 257.78$, $p < 0.001$) and RPE ($F_{3.66, 43.90} = 140.48$, $p < 0.001$) as these variables significantly increased up to $89 \pm 4\%$ $\text{HR}_{\max}$ and 17 RPE during SAFT⁵ ($p < 0.001$) and up to 60% $\text{HR}_{\max}$ and 11 RPE 5 min post-SAFT⁵ ($p < 0.001$).

$\text{JumpHeight}_{\max}$ significantly reduced ($F_{3.41, 47.80} = 39.33$, $p < 0.001$) (● Fig. 2) with a reduction during SAFT⁵ by 5.8 ± 2.0 cm (15%, $p < 0.05$) and after 20 and 30 min passive rest by 1.9 ± 0.1 cm (5%) ($p = 0.019$ and $p = 0.011$).

A significant main effect of SAFT⁵ on H_{ecc} was found ($F_{2.27, 38.29} = 11.01$, $p < 0.001$) (● Fig. 3) with a significant reduction at POST0 (7%, $p = 0.029$), POST15 (13%, $p < 0.001$) and POST30 (18%, $p < 0.001$) compared to PRE, and at POST30 compared to POST0 (12%, $p = 0.024$). This caused a significant main effect in the H_{ecc}/Q_{con} ($F_{2.01, 28.14} = 4.27$, $p = 0.024$). There was no significant reduction in the H_{ecc}/Q_{con} immediately post-SAFT⁵, however, POST15 and POST30 showed a significant reduction of 9% ($p = 0.010$) and 13% ($p = 0.003$).

There was no significant effect of SAFT⁵ on H_{con}/Q_{con} ($F_{2.93, 41.01} = 0.63$, $p = 0.566$). Even though there was a significant effect of SAFT⁵ on both H_{con} ($F_{2.82, 39.42} = 3.91$, $p = 0.025$) and Q_{con} ($F_{1.83, 25.66} = 3.70$, $p = 0.042$) there was only a significant reduction between PRE and POST30 in H_{con} (7%, $p = 0.014$) and Q_{con} (5%, $p = 0.024$) and between POST0 and POST30 in H_{con} (6%, $p = 0.013$) and Q_{con} (4%, $p = 0.045$).

Results of the SLH indicated that SAFT⁵ induced (1) a significantly increased $\text{VGRF}_{\text{peak}}$ between PRE and POST15, POST30 and between POST0 and POST15, POST30, (2) a significantly more extended knee ($2.2 \pm 2.6^\circ$) between PRE and POST30 and (3) a significantly increased internal rotation angle between PRE and POST0 ($4.1 \pm 4.5^\circ$) and POST15 ($4.5 \pm 3.8^\circ$) (● Table 3). No significant effect of SAFT⁵ was found on peak knee-abduction moment in SLH and on any of dependent variables in DVJ. Supplementary files include kinematics and kinetics of an SLH (Appendix 1, 2).

Table 3 Mean $\pm$ SD peak vertical ground reaction force (VGRF_{peak}), knee joint rotations at initial contact (IC), and peak knee abduction moments during single-leg hop (SLH) and drop vertical jump (DVJ) prior to SAFT⁵ (PRE), immediately after (POST0), after 15 min passive rest (POST15) and after 30 min passive rest (POST30).

	SLH			F	PRE	DVJ			F
	PRE	POST0	POST15	POST30		POST0	POST15	POST30	
GRF (N)									
VGRF _{peak}	1857 $\pm$ 325	1864 $\pm$ 375	2099 $\pm$ 363 **	2027 $\pm$ 395 **	860 $\pm$ 246	898 $\pm$ 286	914 $\pm$ 257	930 $\pm$ 266	F _{2,03} , 26.39 = 1.38
knee angle (°)									
flexion (-)	-13.7 $\pm$ 4.7	-11.5 $\pm$ 5.9	-11.3 $\pm$ 4.4	-11.4 $\pm$ 4.7 *	-32.4 $\pm$ 8.6	-29.3 $\pm$ 8.7	-28.6 $\pm$ 8.2	-28.7 $\pm$ 10.2	F _{2,14} , 29.89 = 2.44
internal rotation (+)	-0.5 $\pm$ 6.5	3.6 $\pm$ 7.7 *	3.6 $\pm$ 7.6 *	2.6 $\pm$ 8.0	-2.6 $\pm$ 5.4	-0.8 $\pm$ 6.9	-1.6 $\pm$ 6.9	-1.1 $\pm$ 6.2	F _{1,42} , 19.82 = 0.85
Knee Moment (Nm.kg ⁻¹)									
abduction (+)	0.30 $\pm$ 0.26	0.29 $\pm$ 0.25	0.36 $\pm$ 0.26	0.34 $\pm$ 0.26	0.36 $\pm$ 0.14	0.44 $\pm$ 0.16	0.40 $\pm$ 0.15	0.39 $\pm$ 0.15	F _{1,98} , 27.58 = 0.80

Note * denotes statistically significant differences between marked POST-test and PRE (p < 0.05). ** denotes statistically significant differences between marked POST-test and both PRE- and POST0. § denotes statistically significant main effect over time (p < 0.05).

Discussion

This study determined whether the SAFT⁵, a high-intensity exercise protocol, could be effectively used to induce changes in the muscular and biomechanical characteristics that are typically associated with ACL injury risk. The results partly confirmed the hypothesis. Most notably, there was a significant reduction in H_{ecc} and H_{ecc}/Q_{con}, and altered landing strategies in SLH. The protocol caused a 15% drop in jump performance and RPE was rated between 'hard' and 'very hard' with the average HR (89 $\pm$ 4% HR_{max}) similar to that of female football match play (87–97% HR_{max} [24]). The intensity of the current protocol therefore appears to induce physiological responses similar to those in longer duration match-play situations. In general, current findings imply the importance of screening athletes after a high-intensity functional exercise protocol such as SAFT⁵.

The significant reduction in H_{ecc} immediately after SAFT⁵ and POST15 is in agreement with previous studies investigating the effect of a soccer-specific field test over 90 min (SAFT⁹⁰) [17,43]. The selective occurrence of fatigue in H_{ecc} has been explained previously by the presence of more fatigable type-2 muscle fibres [14], and the high eccentric requirements of the hamstrings in repetitive sprinting and kicking [53] to counteract the anterior shear forces created by the quadriceps. Despite the significant reduction in H_{ecc} between PRE and every post-test, H_{ecc}/Q_{con} was only significantly reduced POST15 and POST30 [17,43]. It should be especially noted that at POST30, H_{ecc}/Q_{con} fell below the at-risk threshold of 0.71 [52]. The delayed reduction of H_{ecc}/Q_{con} can be explained by the short-term high-intensity characteristics of SAFT⁵. Firstly, the occurrence of post activation potentiation (PAP) (i.e., improved muscular performance in response to conditioning stimulus [22]) could have interfered with the effects immediately following SAFT⁵, as previous work has found an improvement in peak force within the first 5 min following exercise [46]. H_{ecc}/Q_{con} is, however, not increased post-SAFT⁵, which assumes that PAP is not the main contributor. Secondly, it could be suggested that there is a delay in peripheral fatigue (i.e., fatigue occurring within the muscle itself) immediately post-SAFT⁵ as a recent study concluded that central fatigue (i.e., alterations in the nervous system) manifests prior to reductions in maximal knee-flexor torque in response to SAFT⁹⁰ [30]. This finding was explained by the earlier and more pronounced effect of central fatigue on explosive force-producing exercises. The delayed onset of peripheral fatigue is represented in the significant reduction in H_{ecc}/Q_{con} after 15 min passive rest and is in agreement with previous research [17,26,43]. There was no significant reduction in H_{con}/Q_{con} post-SAFT⁵ which further supports the use of H_{ecc}/Q_{con} for the evaluation of hamstring function during dynamic activities. Based on H_{ecc} and HQ ratios, it could be assumed that SAFT⁵ induces detrimental effects on muscle performance similar to changes after match-play exertion as SAFT⁹⁰. Detrimental effects are, however, best observed with a 15-min delay.

In accordance with the hypothesis, SAFT⁵ altered landing strategies in the non-dominant leg of an SLH. The reduced knee-flexion angle and increased knee internal rotation are in accordance with other studies exploring the effect of a short-term fatigue protocol on sidestep and stop-jump tasks [8,10,11]. The significant increase in VGRF_{peak} post-SAFT⁵ is in contrast with a previous study investigating the effect of a short-term fatigue protocol [11]. It should be noted that SAFT⁵ aimed to replicate match-play exertion and deviated from the mentioned short-term protocols

by the lack of maximum exhaustion, which increases the stress applied on the body and may cause greater detrimental effects on landing strategies. Several possible speculations could relate to the significantly reduced knee-flexion angle only after 30 min passive rest: (1) the suggested relationship between exercise-related changes in fatigue (e.g., decreased landing forces) and knee laxity (e.g., greater knee-extensor loads and knee shear forces), which is dependent on the baseline knee laxity [42], (2) altered muscular activation patterns in response to fatigue as reduced pre-activation of the hamstrings and gastrocnemius could affect the sagittal and transverse plane differently [15], and (3) post-activation potentiation, as described previously, could play a role [46]. Further studies that take these variables into account will need to be undertaken. Maximum exhaustion may alter landing strategies, which are more related to reduced power generation than to altered stabilization mechanisms. This may explain the different findings of the $VGRF_{peak}$. Previous studies found increased peak knee internal rotation post-fatigue [5,31,40,47] but to date, no study reported increased knee internal rotation at IC during an SLH. Nevertheless, an extended knee position at IC during landing has been associated with increased ACL loading especially during single-leg landing [25]. Additionally, increased internal rotation of the tibia [13,29] and increased $VGRF_{peak}$ [7] have been associated with increased ACL loading. In summary, SAFT⁵ induced kinematic changes in landing strategies of an SLH which are thought to be associated with increased ACL injury risk. This evidence is in support of the notion that biomechanical screening after a functional exercise protocol such as SAFT⁵ may be better suited to identify at-risk individuals than observations without prior high-intensity functional exercises. This was evident in an SLH and not in a bilateral DVJ.

Several limitations to the present study need to be acknowledged. Firstly, due to short-term characteristics of SAFT⁵, different physiological processes will be triggered compared to long-duration match play. More specifically, high-intensity exercises (5 min) induce accumulation of metabolites and ions resulting in metabolic acidosis [48], whereas long-duration exercises impose a greater aerobic demand and may deplete energy supply and cause dehydration [24]. This results in different loading on the body. Secondly, as training status was reported to influence fatigue-induced mechanisms [4], findings of the present study investigating recreationally active athletes cannot be generalised to elite athletes. Athletes with a higher percentage of type-1 fibres (i.e., slow/fatigue resistant fibres) and a higher aerobic fitness are suggested to have, for instance, a greater capability of resisting fatigue [4]. Finally, whilst our study involved a closer simulation of match play than maximal exhaustion protocols may do, its findings do not directly pertain to the effects of actual match play.

The present study was designed to determine the effect of SAFT⁵ on muscular and biomechanical markers of ACL injury risk, primarily to inform the development of future prospective studies that aim at including screening after functional exercises. SAFT⁵ reduced H_{ecc} and H_{ecc}/Q_{con} and caused altered landing patterns in SLH. All of the above have previously been associated with increased ACL injury risk and suggest that testing after a high-intensity functional exercise protocol such as SAFT⁵ may strengthen the identification of individuals with increased ACL injury risk within a sporting population.

Conflict of interest: The authors have no conflict of interest to declare.

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Appendix

Appendix 1 Ankle, knee and hip angles ($^{\circ}$) (mean $\pm$ SD) of sagittal, frontal and transversal plane during stance phase (initial contact + 1 s) of a single-leg hop pre and immediately post SAFT5. Black solid line represents PRE and red solid line represents POST.

Appendix 2 Ankle, knee and hip moments ($\text{Nm} \cdot \text{kg}^{-1}$) (Mean $\pm$ SD) of the sagittal, frontal and transversal plane during the stance phase (initial contact + 1 s) of a single-leg hop pre and immediately post SAFT⁵. Black solid line represents PRE and red solid line represents POST.