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Original research

Thigh muscle activation patterns and dynamic knee valgus at peak ground reaction force during drop jump landings: Reliability, youth competitive alpine skiing-specific reference values and relation to knee overuse complaints

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ABSTRACT

Objectives: (1) To evaluate the reliability of quantifying thigh muscle activation patterns and dynamic knee valgus during drop jump landings, (2) to provide reference values for female and male youth alpine skiers, and (3) to study their associations with age, anthropometrics, biological maturation and knee overuse complaints.

Design: Cross-sectional biomechanical experiment including questionnaires.

Methods: One hundred fourteen skiers of the under 16 category (main experiment) and twelve healthy participants (reliability experiment) volunteered. Quadriceps-to-hamstring-activation ratio and medial knee displacement at peak ground reaction force during drop jump landings were measured using marker-based motion analysis, force plates and electromyography. Additionally, age, anthropometrics, biological maturation and knee overuse complaints were assessed.

Results: There were good test–retest reliabilities and moderate standardized typical errors for both quadriceps-to-hamstring-activation ratio (intraclass correlation coefficient(3,1) = 0.84 [95% confidence interval: 0.69, 0.94]; standardized typical errors = 0.43 [0.35, 0.56]) and medial knee displacement (intraclass correlation coefficient(3,1) = 0.87 [0.74, 0.95]; standardized typical errors = 0.39 [0.32, 0.50]). Male skiers had a significantly higher quadriceps-to-hamstring-activation ratio (3.9 ± 2.0 vs. 2.9 ± 1.4 , $p = 0.011$), whilst medial knee displacement was comparable to females ($12 \text{ mm} \pm 11 \text{ mm}$ vs. $13 \text{ mm} \pm 9 \text{ mm}$; $p = 0.419$). In male skiers, medial knee displacement correlated with anthropometrics and maturity offset; in female skiers, quadriceps-to-hamstring-activation ratio and medial knee displacement were associated with knee overuse complaints ($p < 0.05$).

Conclusions: Female and male youth skiers use different thigh muscle activation strategies, but show comparable dynamic knee valgus motions during drop jump landings. In females, a combination of increased relative quadriceps activity and medial knee displacement may favour knee overuse complaints.

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Practical implications

- Our biomechanical approach provides a reliable assessment method to quantify the activation ratios of the thigh muscles and dynamic knee valgus motion under highly dynamic loading conditions such as a drop jump (DJ) landings.

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- Female and male youth competitive skiers employ different thigh muscle activation strategies during DJ landings, but show comparable dynamic knee valgus motions.
- Quadriceps dominant muscle activity coupled with dynamic knee valgus motion may favour the occurrence of knee overuse complaints in female youth competitive skiers. Accordingly, their supplemental preventative training should target the following: (1) increasing leg axis stability; (2) ensuring adequate maximal eccentric hamstring strength (to counteract quadriceps-induced anterior tibial translation); (3) optimising intra- and intermuscular coordination of the thigh muscles (e.g. by plyometric jump training); and (4) developing

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favourable (axially aligned, less stiff, and less quadriceps activating) landing technique.

- The presented work could therefore contribute to the possible identification of female athletes at risk for developing knee overuse complaints and to the derivation of appropriate prevention strategies.

1. Introduction

Competitive alpine skiing is a physically demanding sport that places considerable loads on the skiers' bodies.^{1–3} The high prevalence of traumatic injuries has led to the topic being the subject of research for several years now.^{4–6} In contrast, comparatively little research has been conducted in the area of overuse injuries,⁷ despite the fact that they outnumber traumatic injuries manifold.⁸ With respect to overuse injuries, the knees (and especially the patella tendon) are most affected in both youth and elite skiers.^{9–11} A major concern pertains to youth skiers around the growth spurt, whose bodies are still maturing and, therefore, are especially sensitive to unfavourable loads.^{11,12} Moreover, in under 16 years old (U16) skiers, overuse injuries revealed to be more frequent in females than in males.⁹

In skiing, up to 1.75 times the body weight can act on each leg,¹³ and the knee joint is inevitably subjected to extreme kinematics, including high degrees of flexion and valgus.¹⁴ In addition, due to uneven or bumpy snow surfaces and the chattering of the skis when interacting with the snow surface whilst turning, skiers are typically exposed to high vibration loads and impact-like shocks, that are partially attenuated by the knee joint and the perturbation-induced activation of its stabilizing muscles.^{15,16} Besides the accumulation of such repetitive adverse loads during skiing, the focus of off-snow training for skiers is also on maximal/explosive leg strength training and plyometrics (including extensive amounts of jump training),² adding further mechanical stress to body and major joints.

To date, the exact risk factors and mechanisms leading to knee overuse complaints in skiers are not fully understood, and reasonable protocols to functionally assess athletes at increased risk are widely lacking. However, based on the specific loading patterns of skiers (as described above), some aetiology-based link to the imitation exercise “drop jumps” (DJs) is evident. This is why such movement task may have potential for the purpose of athlete screening; sufficient reliability and clinical relevance provided.

From a biomechanical perspective, there are two independent aspects that may play a functional role for developing knee overuse complaints: lower limb motion in the frontal plane and neuromuscular factors affecting the sagittal plane kinematics. Regarding the first aspect, a recent systematic review and meta-analysis found preliminary evidence associating impaired kinematics during jump landings with lower limb overuse injuries.¹⁷ Generally, unfavourable leg alignment and excessive dynamic knee valgus (i.e. medial knee movement in the frontal plane during motion) have been described to increase the structural load on the musculoskeletal system and consequently lead to knee overuse complaints.¹⁷ Since there is evidence that males and females differ in neuromuscular development from puberty onward, a sex-specific analysis appears to be warranted.^{18,19}

Concerning the second aspect, it is known that the quadriceps (Q) and hamstring (H) muscles serve as the main stabilizers of the knee,²⁰ and that thigh muscle strength deficits and imbalances (i.e. H:Q strength ratios below normal range) may favour the occurrence of overuse injuries in the knee.^{21,22} We suspect that not only the strength ratio is decisive for a predisposition to knee overuse complaints, but that possibly the Q:H activation ratio under high and dynamic loading (such as during a drop jump (DJ)) could provide information about this. Thereby, translational movements of the tibia relative to the femur, as they can theoretically occur due to imbalanced or poorly coordinated Q:H activation patterns,²³ may play a key role for developing knee overuse complaints.

Therefore, the aims of this study were (1) to evaluate the reliability of quantifying thigh muscle activation patterns and dynamic knee valgus at peak ground reaction force during DJ landings, (2) to provide reference values for female and male youth competitive alpine skiers, and (3) to study their associations with age, anthropometrics, biological maturation and the occurrence of knee overuse complaints.

2. Methods

In a first cross-sectional experiment that aimed to investigate the test–retest reliability (hereinafter called “reliability experiment”), 12 healthy and sportive participants were recorded within one day for five DJ trials, in which their thigh muscle activation patterns, as well as their knee valgus motion were assessed. Participants were on average 28.6 ± 4.7 years old, with a body height of 171.2 ± 8.4 cm and a body weight of 66.6 ± 9.8 kg. Except for the number of repetitions of valid trials, the instructions were identical to the “main experiment” (described below), as was the measurement setup used. Participants were recruited through a public call and were eligible if fulfilling the inclusion criterion of regular physical activity. None of them met the exclusion criteria, i.e. age under 18 or over 40, BMI over 45 or reduced load tolerance due to injuries or pathologies.

In a second cross-sectional experiment (i.e. the “main experiment”), 114 youth competitive alpine skiers of the under 16 (U16) category (47 females, 67 males) were tested for their thigh muscle activation patterns and dynamic knee valgus motion during DJ landings. The main experiment was complemented by assessing age and anthropometric measures. Based on this data, skiers' biological maturation (i.e. the age at peak height velocity (APHV [years]), as well as the maturity offset (MO [years]) as the point in time at which the athlete is currently in his or her development relative to the APHV) were estimated using the Mirwald-formula.²⁴ Moreover, the occurrence of substantial knee overuse complaints within the 12 months prior to the main experiment was surveyed by the use of (i) the Oslo Sports Trauma Research Centre (OSTRC) questionnaire on health problems,²⁵ and (ii) retrospective supplementary interviews directly before the experiment, a methodological procedure described in more detail elsewhere.¹¹ The division into substantial and non-substantial knee complaints was based on the OSTRC severity score, where *substantial* defines as moderate or severe reductions in training volume, moderate or severe reductions in sports performance, or complete inability to participate in sport.²⁵ On average, males were 0.2 years older than females (14.9 ± 0.5 years vs. 14.7 ± 0.6 years). Whilst being of comparable body weight and BMI, males were on average 5.4 cm taller, but less progressed in their biological development (1.5 years behind in their maturity offset) compared to females, see Table 1. All participants were members of a youth development structure of the Swiss National Skiing Association (Swiss-Ski). Eligible skiers had to meet the criterion that they were not in a back-to-sports program after an injury and did not have systematic pathologies, diabetes mellitus, or inflammatory arthritis. No exclusion was made based on these study eligibility criteria.

The protocols underlying both experiments were approved by the cantonal ethics committee Zurich (KEK-ZH-NR: 2017-01395) and all participants provided written informed consent. All data collection and processing were conducted in accordance with the Declaration of Helsinki and national laws.

To perform the DJ task, participants were instructed to drop down barefoot, with their hands placed at their hips, from a 32 cm high box before performing a jump with shortest possible contact time and greatest possible jump height. An eight-camera optoelectronic motion capture system (Vicon, Oxford Metrics, UK) allowed tracking the movements of the skin marker-equipped participants. With this method, the camera emits an infrared light that is reflected by the markers and recaptured by the camera. Coupled with the information about the position of the cameras relative to each other, the exact marker position in the measurement volume can be identified. The system operated at

Table 1
Baseline characteristics for the participating youth competitive alpine skiers of the under 16 (U16) category.

	Female U16 skiers (n = 47)	Male U16 skier (n = 67)	p-Value	Effect size r
Age [years]	14.7 ± 0.6 [13.5; 15.9]	14.9 ± 0.5* [13.8; 15.8]	0.032	0.20
Body height [cm]	162.6 ± 5.4 [148.0; 171.0]	168.0 ± 8.1*** [150.5; 187.0]	0.000	0.38
Body weight [kg]	54.2 ± 7.6 [38.0; 74.0]	56.6 ± 10.0 [36.0; 85.0]	0.138	0.14
BMI [kg/m ²]	20.4 ± 2.3 [15.0; 25.6]	19.9 ± 2.2 [15.2; 25.5]	0.176	0.13
Maturity offset [years]	2.1 ± 0.6 [1.0; 3.2]	0.6 ± 0.9*** [−1.8; 2.3]	0.000	0.72

All data are presented as mean ± SD and [min.; max.]. Level of significance based on unpaired samples *t*-tests and backed-up by bias corrected accelerated (BCa) bootstrapping with 10,000 samples: ****p* < 0.001, **p* < 0.05; BMI: body mass index.

200 Hz and with a measurement accuracy of ~1 mm, which was ensured by frequent calibrations. The modified PlugIn-Gait marker set used, as well as the joint centre determination and customized Matlab Scripts (Matlab, The MathWorks, Inc., US), corresponded to the procedure described in a previous study.¹⁹ Additionally, two force plates (SP Sportdiagnosegeräte GmbH, Austria) as well as an eight electrode electromyographic system (Aktos, Myon AG, Switzerland) recorded the DJ tasks at 2000 Hz. All systems were integrated into the Vicon system and thus synchronised. Ground reaction force data were filtered using a zero-lag Butterworth low pass filter with a cut-off frequency of 200 Hz. Maximum voluntary contraction (MVC) for the quadriceps muscle of each leg was determined whilst sitting on a chair and maximally pushing against a fixed ankle cuff resistance for at least 5 s. The hamstring MVC was assessed in a kneeling position (with the shoulder, hip and knee forming a straight line) on a Nordic hamstring exercise device (Nordbord, Vald Performance, Australia), by maximally pushing against an examiner induced constant forward pressure applied to the hip and shoulders. EMG signal artefacts, defined as values higher than five times the standard deviation of the raw signal, were detected and removed. EMG data were then rectified and filtered with a 4th order zero-lag Butterworth filter and bandpass filter with cut-off frequencies of 20 Hz and 500 Hz. The filtered data was smoothed by a point-by-point symmetric moving 30 ms root mean square. Muscle activations during DJ landings were normalized to the corresponding isometric MVC trials for each muscle separately, whereby the averaged 3 s with highest activation provided the 100% MVC and the lowest average 3 second activation the baseline.

Participants' dynamic knee valgus motions during DJ landings were quantified as the medial knee displacement (MKD), i.e. the rectangular medial distance [mm] of the knee joint centre relative to a reference plane formed by the hip, knee and ankle joint centres one frame before the initial ground contact.¹⁹ However, in contrast to our previous study,¹⁹ MKD at the moment of maximum vertical ground reaction force (MKD_{GRF}) (and not the maximum MKD amplitude) was considered being the most relevant metric for further analysis. The corresponding values were averaged over the two landings and both legs. High muscle activation patterns during DJ landings were quantified as the quadriceps-to-hamstring (QH) ratio, i.e. the percentage activation of two added-up quadriceps muscles (M. vastus medialis and M. vastus lateralis) divided by the sum of two hamstring muscles (M. semitendinosus and M. biceps femoris). The QH_{GRF} was consequently the QH at the time point of the maximum vertical GRF. Analogue to the MKD_{GRF}, the corresponding values were averaged over both landings and both legs.

The data analysis of the reliability experiment was carried out in accordance to the guidelines of Hopkins,²⁶ and analogous to previous reliability evaluations in which the procedure was described in detail.²⁷ It was taken into account for the habituation effect occurring in

performance testing by using the open access spreadsheets from Hopkins,²⁸ and the intraclass correlation coefficient ICC(3,1) as well as the within-subject error, also referred to as standardized typical error, were calculated. To obtain these values, the DJ landing tasks were carried out five times within one day by each participant and subsequently evaluated for MKD_{GRF} QH_{GRF}. The ICC values were classified based on the recommendation of Koo et al.²⁹ (<0.5, *poor*; 0.5 to 0.75, *moderate*; 0.75 to 0.9 *good*; and >0.9 *excellent* reliability). To enable a categorisation of the standardized typical errors, their values were doubled before interpretation (0.2, *small*; 0.6, *moderate*; 1.2, *large*; 2.0, *very large* and 4.0, *extremely large*).³⁰

The statistical analysis of the main experiment was performed in SPSS (Version 26, IBM Corporation, US). First, the data were assessed for normal distribution using Shapiro–Wilk tests, shape parameters and graphical techniques such as histograms. In cases, where analysed (subgroup-)data did not substantially deviate from normal distribution, namely skewness > 2 and kurtosis > 7, according to West et al.,³¹ bias-corrected and accelerated (BCa) bootstrapping with 10,000 samples was applied. If the normal distribution was not given, non-parametric tests were used. Participants' age, anthropometrics, biological maturation and DJ analysis-related measures (QH_{GRF} and MKD_{GRF}) were expressed as *mean* ± *SD*. To compare age, anthropometrics, and biological maturation between the male and female groups, independent *t*-tests (*p* < 0.05, backed-up by bias corrected accelerated bootstrapping (BCa) with 10,000 samples) were applied and, corresponding effect sizes *r* were calculated. For analysing sex differences between QH_{GRF} and MKD_{GRF}, Mann–Whitney *U* tests (*p* < 0.05) were used and effect sizes *r* were reported. The relationship between the anthropometrics- and maturation-related baseline characteristics and MKD_{GRF} as well as QH_{GRF} was assessed using Spearman's rank correlation coefficients (*p* < 0.05). The association of MKD_{GRF} and QH_{GRF} with the occurrence of substantial knee overuse complaints [yes; no] was tested by means of binary logistic regression (*p* < 0.05), backed-up by BCa bootstrapping with 10,000 samples.

3. Results

The test–retest reliability of both QH_{GRF} and MKD_{GRF} revealed *good* test–retest reliability and *moderate* standardized typical errors. Regarding QH_{GRF}, a repeated measures ANOVA over five trials within the same group of twelve healthy participants resulted in an ICC(3,1) of 0.84 [95% CI: 0.69, 0.94] with a standardized typical error of 0.43 [95% CI: 0.35, 0.56]. The corresponding within-subject standard error of measurement (SEM) was 0.51 [95% CI: 0.42, 0.66]. For the equal evaluation of the MKD_{GRF} the ICC(3,1) was 0.87 [95% CI: 0.74, 0.95], with the standardized typical error of 0.39 [95% CI: 0.32, 0.50] and a within-subject SEM of 4.4 mm [95% CI: 3.6 mm, 5.7 mm].

The detailed descriptive and inferential statistics of MKD_{GRF} and QH_{GRF} in female and male U16 skiers are presented in Table 2. The entire time courses of MKD and QH during all functional phases of the DJ task are illustrated in the Supplementary material (Files 1 and 2). MKD_{GRF} values showed an overall average of 12 mm ± 10 mm and there were no sex differences at *p* < 0.05. The average QH_{GRF} ratio over both sexes was 3.5 ± 1.9, representing a more than three times higher

Table 2
Medial knee displacement (MKD_{GRF}) and quadriceps-to-hamstring muscle activation ratio at maximal ground reaction force (QH_{GRF}) in youth competitive alpine skiers of the under 16 (U16) category.

	Female U16 skiers (n = 47)	Male U16 skiers (n = 67)	p-Value	Effect size r
MKD _{GRF} [mm]	13 ± 9 [1; 35]	12 ± 11 [1; 59]	0.419	0.08
QH _{GRF} [-]	2.9 ± 1.4 [0.7; 6.8]	3.9 ± 2.0* [1.1; 9.7]	0.011	0.24

Data are presented as mean ± SD and [min.; max.]. Level of significance based on Mann–Whitney-Utests: **p* < 0.05; BMI: Body Mass Index.

Table 3

Binary logistic regression analysis assessing the association of medial knee displacement at the maximal ground reaction force (MKD_{GRF}) and the quadriceps-to-hamstring muscle activation ratio at the maximal ground reaction force (QH_{GRF}) with the occurrence of substantial knee overuse complaints in female youth competitive alpine skiers of the under 16 (U16) category.

Model parameter	Dependent variable	Independent variable	B	e^B	SE_B	p-Value ^a
Female U16 skiers (n = 47) Chi-Square = 17.546 p = 0.000 $R^2_{Nagelkerke}$ = 0.583 Cohen f^2 = 1.400	Substantial knee overuse complaints [yes; no]	MKD_{GRF} [mm]	0.169	1.184	0.079	0.032*
		QH_{GRF} [-]	1.447	4.250	0.543	0.008**

^a Level of significance backed up by bias-corrected and accelerated (BCa) bootstrapping with 10,000 samples: *p < 0.05, **p < 0.01.

activation of the quadriceps muscle in relation to the hamstring muscles at the point of maximal ground reaction force, with male U16 skiers having significantly higher QH_{GRF} values at $p < 0.05$.

Assessing the relationship between MKD_{GRF} , age, anthropometric measurements, and biological maturation, respectively, some significant inter-parameter correlations were found in the group of males, but not in females. MKD_{GRF} of males correlated most strongly with the maturity offset ($p = 0.005$, $\rho = 0.342$), whereas slightly weaker correlations were found for body weight ($p = 0.007$, $\rho = 0.329$), body height ($p = 0.009$, $\rho = 0.318$) and BMI ($p = 0.029$, $\rho = 0.267$), but none for age ($p = 0.339$, $\rho = 0.119$). QH_{GRF} , in contrast, did not show any significant correlations with age, anthropometrics, or maturation in neither of the groups at $p < 0.05$.

Within the 12 months preceding the experiment, 17 of the 114 U16 skiers (14.9%) reported to have suffered from substantial knee overuse complaints. The rate for female youth skiers was 12.8%, and the rate for males was 16.4%. A binary logistic regression analysis in female skiers (Table 3) revealed significant associations between the occurrence of substantial knee pain [yes; no] and MKD_{GRF} and QH_{GRF} (Chi-Square = 17.546, $R^2_{Nagelkerke} = 0.538$, $p = 0.000$, $n = 47$). Odds ratios indicate, that if MKD_{GRF} and QH_{GRF} increase by one unit each, the relative probability of the occurrence of substantial knee complaints increases by 18.4% and 325%, respectively. For males, the same independent variables (MKD_{GRF} ; QH_{GRF}) were not significantly associated with knee overuse complaints (Chi-Square = 2.343, $R^2_{Nagelkerke} = 0.058$, $p = 0.310$, $n = 67$).

4. Discussion

As shown in the reliability experiment, QH_{GRF} as well as the MKD_{GRF} during vertical DJ landings can be reliably quantified with *good* test-retest reliability and *moderate* typical errors. The within-subject standard error of measurement can be considered acceptable with an observed value three to four times smaller compared to the corresponding standard deviation of the parameter. From this point of view, a biomechanical test approach as presented here, may be sensitive enough to detect clinically relevant differences and could represent a reasonable alternative to on-slope measurements in skiing, in which the number of non-controllable influencing factors may accumulate.

The main experiment revealed that MKD_{GRF} was of comparable magnitude between male and female U16 skiers, whereas QH_{GRF} was significantly higher in the group of males. The comparability in MKD between the sexes, seems surprising at first glance, since dynamic knee valgus motion is commonly described as a problem affecting female athletes.³² If the biological age difference between the sexes is considered, however, a different picture emerges: MKD_{GRF} and the maturity offset positively correlate in the group of males who were close to their APHV and therefore are thought to produce a higher MKD_{GRF} . Female skiers, in contrast, were already past their APHV, but still had comparably high values although their neuromuscular system should have already adapted to the developmental changes. Generally, MKD_{GRF} appears before the maximum MKD (File 2), which explains the lower values compared to a previous study reporting maximum MKD .¹⁹ Regarding the increased QH_{GRF} of male skiers, it can be seen from the Supplementary material (File 1) that the increased ratio in males does not

result from pronounced quadriceps activity, but rather from reduced activity of the hamstring muscles. This indicates that male skiers might use a less conservative landing strategy than females, putting them at supposedly higher risk for overuse injuries. However, this seems not to be the case, as high QH_{GRF} was only found to be associated with knee overuse complaints in females, who showed an excessive quadriceps-activity during DJ landings.

Transferred to the ski setting, this implies that both male and female skiers repeatedly compensate for high loads with unfavourable muscle activation and knee position, burdening their knee joints.³³ Males (in our study around their growth spurt) might improve their dynamic knee control by neuromuscular adaptation after their rapid growth spurt. A relatively short period of unbalanced thigh muscle activation thus might not have a quantifiable influence on the development of knee overload complaints. Contrary, in females (already past their growth spurt) a clear association between QH_{GRF} , MKD_{GRF} and the occurrence of knee overuse complaints was found, possibly putting them at risk for future degenerative joint diseases. As knee overuse complaints mostly have gradual onset¹⁷ we suspect that subliminal inconsistencies of the locomotor system, when present over time, may lead to overuse complaints. If the imbalances manifest, as we saw in females, they might trigger long-term overuse complaints. Our results, and the high number of youth skiers suffering from substantial knee overuse complaints, indicate that the growth spurt is a sensitive developmental phase in which increasing training load does not necessarily contribute to improved performance, but may lead to injury-related absences in training and/or competition.

Some methodological limitations should be considered in the interpretation of the present data. Because of athlete availability and time constraints when testing large cohorts of athletes within their real training settings and facilities, the reliability assessment was conducted in a separate experiment with a different sample. With regard to practical relevance, caution is required when transferring the results from the biomechanical test setting to the on-snow situation in alpine skiing, as the neuromuscular patterns of DJ landing do not comprehensively replicate those of skiing.³⁴ Furthermore, a global parameter was chosen to determine the medial knee displacement and a ratio was used to describe muscle activation. Global parameters cannot provide breakdowns into individual components such as hip adduction and internal knee rotation in the case of MKD_{GRF} . A ratio, on the other hand, does not consider the absolute activation, which can lead to misleading conclusions if solely the ratio is factored. Both parameters, however, describe a functional interaction that seems to become relevant for the development of complaints when they occur in combination. Future research therefore should focus on the decomposition of the individual factors.

5. Conclusions

Female and male youth competitive alpine skiers use different thigh muscle activation strategies during DJ landings whilst showing similar dynamic knee valgus motions. In female skiers, excessive relative quadriceps activity coupled with medial knee displacement may increase the

likelihood of suffering knee overuse complaints. The results furthermore highlighted that, gender- and biological maturation-specific research on the emergence of knee overuse complaints should continue to be pursued, as well as the development of targeted prevention programmes.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jsams.2021.06.006>.

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Declaration of interest statement

None declared.

Confirmation of ethical compliance

The study protocol was approved by the cantonal ethics committee Zurich (KEK-ZH-NR: 2017-01395) and is in conformity with the Helsinki Declaration and national laws. All participants signed an informed consent form. If they were younger than 14 years, their legal guardians signed instead.

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