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CLINICAL SCIENCE

Isokinetic analysis of ankle and ground reaction forces in runners and triathletes

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OBJECTIVE: To analyze and compare the vertical component of ground reaction forces and isokinetic muscle parameters for plantar flexion and dorsiflexion of the ankle between long-distance runners, triathletes, and non-athletes.

METHODS: Seventy-five males with a mean age of 30.26 ($\pm$6.5) years were divided into three groups: a triathlete group (n = 26), a long-distance runner group (n = 23), and a non-athlete control group. The kinetic parameters were measured during running using a force platform, and the isokinetic parameters were measured using an isokinetic dynamometer.

RESULTS: The non-athlete control group and the triathlete group exhibited smaller vertical forces, a greater ground contact time, and a greater application of force during maximum vertical acceleration than the long-distance runner group. The total work (180°/s) was greater in eccentric dorsiflexion and concentric plantar flexion for the non-athlete control group and the triathlete group than the long-distance runner group. The peak torque (60°/s) was greater in eccentric plantar flexion and concentric dorsiflexion for the control group than the athlete groups.

CONCLUSIONS: The athlete groups exhibited less muscle strength and resistance than the control group, and the triathletes exhibited less impact and better endurance performance than the runners.

KEYWORDS: Stress Fracture; Tibia; Run; Triathlon.

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INTRODUCTION

In long-distance aerobic sports such as running and the triathlon (1,2), the lower limbs are often the sites of overload injuries. Stress fractures of the tibia are prominent among such injuries (3). Epidemiological studies on recreational and competitive runners have shown that, over a one-year period, more than 50% of athletes incur tibia stress fractures (2,4). Investigations focusing on the injury rate during triathlons have indicated that such injuries occur mainly during pre-competition training periods preceding important championships (2,5).

These injuries develop through repetitive overloading, which alters bone homeostasis, thus increasing osteoclastic activity accompanied by inadequate repair (6). The etiological factors may be intrinsic (anatomical, biomechanical, or demographic) or extrinsic (characteristics relating to training) (7). Muscle fatigue around the ankle has been correlated with the physiopathology of stress fractures in sports that involve running (8) due to the loss of the eccentric contraction capacity of the dorsiflexors during heel strike (9) caused by a decline in proprioception of the mechanical stress on the cortical bone layer (1). This effect compromises the capacity of these muscles to dissipate impact forces (10).

Although several studies have shown that the impact force exerted by individuals with a history of tibia stress fractures is greater than the force exerted by individuals without such a history (1,11), data that can be used to characterize the tibial musculature are scarce. This musculature plays an essential role in attenuating these ground reaction forces. A study in 2009 suggested that deficits in tibial musculature strength might contribute to the etiology of stress fractures. The study examined a group of long-distance runners with previous tibia stress fractures who presented lower leg musculature with a smaller cross-sectional area than a group without a history of fractures. However, the study indicated that a more quantitative assessment was needed to confirm these results (12).

No potential conflict of interest was reported.

The dynamic fatigue performance of muscles can be assessed using isokinetic dynamometry, a method that determines the functional pattern of muscle strength and equilibrium (13). Impact is generally assessed on a force platform by analyzing the first peak of the vertical component of the ground reaction force (14,15). Although the second peak or active peak has a longer duration and is a lower-frequency component, it also has an important role in the evaluation of the kinetic relationships to overuse injuries (16).

Studies have focused on muscle and kinetic parameters and their possible relationships with the incidence of tibia stress fractures in groups with a recent history of injury, rather than examining the pre-fracture period. Moreover, most studies have given priority to long-distance runners (1,8,11,12) and have made no comparisons with other sports that also result in fractures. Therefore, studying the muscle and kinetic characterization of vertical ground reaction forces among triathlon athletes and long-distance runners, and comparing them with non-athletic individuals who are not subjected to high training loads, is important for developing possible strategies to prevent overuse injuries in these two sports. Such strategies would be complementary to strategies for controlling other factors (both extrinsic and intrinsic) relating to tibia stress fractures. Thus, the present study had the objective of comparing long-distance runners, triathletes and non-athletic individuals in regard to the strength and endurance of their dorsiflexion and plantar flexion ankle musculature, as measured by means of isokinetic dynamometry, along with the measurement of the vertical component of their ground reaction force, by means of a force platform.

METHODS

The study was performed at the Laboratory for the Study of Movement, Institute of Orthopedics and Traumatology, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. The study was approved by the Ethics Committee of the University of São Paulo (n° 932/08).

Subjects

A total of 75 males with a mean age of 30.26 ± 6.51 years, a mean height of 1.74 ± 0.06 m, and a mean weight of 71.26 ± 9.41 kg were recruited for this study. These patients were divided into three groups: a triathlete group (TG) composed of 26 triathletes who had regularly trained for competition for at least one year (6.5 ± 5.6 years) and had a weekly training regimen (homogeneous in the three months prior to the evaluations) of at least 30 km of running (50.71 ± 16.04 km), 60 km of cycling (230.76 ± 84.1 km), and 5 km of swimming (9.32 ± 4.11 km); a long-distance runner group (LDRG) composed of 23 long-distance runners who had regularly trained for competition for at least one year (6.5 ± 5.6 years) and had a weekly training regimen (homogeneous in the three months prior to the evaluations) of at least 60 km of running (104.23 ± 36.89 km); and a control group (CG) composed of 26 non-athletes who did not regularly train for any sports but performed some type of physical activity two to three times per week for at least the three months prior to the evaluations (17). Calculations determined that a sample of 22 subjects in each group allows, with a 90% power, confirmation that differences greater than or equal to a standard deviation are statistically significant at a 5% significance level. The three groups did not have any ankle joint injuries in the six months preceding the study and did not experience pain during the experimental period. Injury was defined as an event that prevented the athlete from training for a sport for 24 or more consecutive hours (2). All of the subjects gave written informed consent.

Procedures

To perform the evaluations, the individuals were scheduled for a single session. The participants were instructed to attend the session dressed in sports attire and regular sports shoes (training shoes for the athletes) (1,15). The individuals were also asked to not perform any high-intensity physical activity in the 12 hours prior to the session. On the day of the evaluation, the individuals signed an informed consent form to participate in the study and answered a questionnaire regarding their years of regular training and their regimen and frequency of training. After the questionnaire, the height and body mass of all of the participants were measured, and the participants were evaluated via a force platform evaluation and isokinetic evaluation.

Force platform evaluation

Ground reaction force data were collected using a force platform (AMTI) connected to an ITAUTEC computer via an A/D converter. The platform was turned on 30 minutes before the start of data collection to verify the amplification, frequency, and signal capture parameters. The frequency was 200 Hz, the time needed for acquisition was 3 s, and these values were calculated with AMTI's BioAnalysis software. The platform (1.2x0.6 m) was fixed on a flat surface and covered by a black rug, which prevented the participant from knowing the position of the platform. The participants were instructed to run a 10.5 m path at an average speed of 3.75 m/s ± 7%. One foot was required to land completely on the platform (located at 5.32 m from the starting point) without a significant alteration in the step (11). This goal was considered to be a practical experience and indicated acquisition success (1,11).

To familiarize the participants with the evaluation process and to register the data, ten trials were performed, which was an appropriate quantity to evaluate the ground reaction force data (18). Of these ten trials, five were performed with the right limb and five with the left (14), and the limbs were recorded randomly by blindly drawing cards (15). The foot that was resting on the platform when the participant started the path was observed. The kinetic variables analyzed were the following: the total time in seconds that the foot was in contact with the ground (ST), the maximum force along the vertical axis (F_z max), the average force along the vertical axis (F_z avg), the force corresponding to the maximum deceleration along the vertical axis (F_z max acceleration), and the time in seconds that the F_z max acceleration occurred (F_z max acceleration@Time). All of the variables related to the vertical ground reaction forces were normalized to body weight (11).

Isokinetic evaluation

To evaluate the isokinetic variables, a Biodex Isokinetic Dynamometer (System 3, Software version 3.2) was used. Prior to the test, the participants were subjected to a warm-up with an ergonomic bicycle for five minutes, which consisted of a submaximal effort (with a comfortable load and a cadence that did not cause fatigue) (17). Then, the dorsiflexor and plantar flexor muscles were stretched in three series of 30 s. Before the start of the tests, the isokinetic dynamometer was calibrated and positioned for optimal performance. The participants were asked to sit, the limb that was tested was placed in a support in the distal portion of the thigh, and the sole of the foot was supported by a rigid plate. The biological axis of motion of the ankle joint was aligned with the mechanical axis of the dynamometer, and the knee was held at 30˚ of flexion. The rigid plate allowed a 20˚ range of plantar flexion from the neutral position of the ankle. The participant was held in this position by two thoracic belts, one pelvic belt, Velcro straps on the distal portion of the thigh, and Velcro straps on the metatarsal area in the dorsal region of the foot. The participants were instructed to hold onto the lateral support (arms) of the chair to improve stability.

After positioning, three submaximal repetitions were performed to familiarize the patient with the equipment. To register the data, a set of five repetitions at a velocity of 60˚/s and another set of 30 repetitions at 180˚/s were completed (19) in the concentric/eccentric mode and eccentric/concentric mode for both plantar flexion and dorsiflexion. Ten seconds of rest were allowed between the sets. All of these tests were bilateral and standardized, with the right lower limb the first to be evaluated. For the duration of the tests, constant verbal encouragement was used to help the participants maintain maximum strength during the contractions. For the speed of 60˚/s, the peak torque was analyzed, defined as the maximum torque obtained for the series of five repetitions, and expressed in Newton-meters (N·m). For the 180˚/s speed, the total work was analyzed, defined as the sum of muscle work performed in the 30 repetitions of the series, and expressed in joules (J).

Statistical Analysis

The values obtained for all of the variables for the three groups are displayed in tables. The normality of these variables was verified by the Kolmogorov-Smirnov test.

The non-dominant and dominant limbs were compared using a t-test for the dependent samples in all of the groups with the objective of observing possible differences between them. The analyses did not discriminate between the limbs because no significant differences were found between them. Regarding the comparisons among the variables with a Gaussian distribution, ANOVA (Analysis of Variance) and Tukey's post hoc tests were used. For non-normal distributions, the Kruskal-Wallis and Müller-Dunn post hoc tests were used. To compare isokinetic performance, Chi-square tests were used to compare the results of eccentric contraction of plantar flexor testing of the three groups at 180˚/s. The statistical software SPSS (Statistical Package for Social Science, version 15.0 for Windows) was used for the analyses, and a value of $p \leq 0.05$ was considered to be statistically significant.

RESULTS

Kinetic Evaluation

To analyze the kinetic variables, a comparison between the triathlete group, the long-distance runner group and the control group was performed (Table 1).

Significant differences were found for all of the variables analyzed. The control group and the triathlete group presented significantly lower averages than the long-distance runner group for the forces along the vertical axis, and they presented significantly higher times corresponding to the period in which the foot was in contact with the ground. The control group presented a significantly higher average for the total time the force corresponded to the maximum acceleration along the vertical axis variable than the triathlon and the long-distance runner groups, and the triathlete group had a significantly higher average compared with the long-distance runner group.

Table 1 - The means (SD) of kinetic variables for the groups and the comparison among the triathlete group, the long-distance runner group, and the control group.

	T Group	LDR Group	C Group	
	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
ST (s)	0.22 (0.03)[a]	0.18 (0.02)[b,c]	0.22 (0.04)[a]	0.000*
Fz Max. (N. N^{-1})	2.56 (0.36)[a]	2.8 (0.19)[b,c]	2.52 (0.24)[a]	0.000*
Fz Avg (N. N^{-1})	1.54 (0.21)[a]	1.68 (0.13)[b,c]	1.5 (0.15)[a]	0.000*
FzMD (N. N^{-1})	2.4 (0.36)[a]	2.57 (0.24)[b,c]	2.31 (0.24)[a]	0.000*
FzMAccl. (N. N^{-1})	1.73 (0.29)[a]	1.99 (0.21)[b,c]	1.8 (0.27)[a]	0.000*
FzMA@Time (s)	1.86 (0.2)[a,c]	1.74 (0.22)[b,c]	1.97 (0.22)[a,b]	0.000*

T, triathletes; LDR, long-distance runners; C, controls; ST, total time of contact with the ground; Fz Max., maximum force along the vertical axis; Fz Avg, average force along the vertical axis; FzMD, force corresponding to maximum deceleration along the vertical axis; FzMAccl., force corresponding to maximum acceleration along the vertical axis; FzMA@Time, time in which Fz Max Acceleration occurs; [a]significantly different from the LDR group; [b]significantly different from the TG = group; [c]significantly different from the C group; *$p<0.05$.

Isokinetic evaluation

To analyze the isokinetic variables for the 60 and 180˚/s velocities, a comparison between the triathlete group, the long-distance runner group, and the control group was performed (Tables 2 and 3).

The peak torque during the eccentric contractions of the plantar flexors and the concentric contractions of the dorsiflexors was higher in the control group than in the triathlete and long-distance runner groups. The control group had higher concentric contraction values than the triathlete group for the 60˚/s velocity.

At a 180˚/s velocity, the total work for the concentric contractions of the dorsiflexors for the control group was greater than that of the TG and LDRG, and the TG work value was greater than the LDRG. The total dorsiflexor eccentric work was greater in the control and triathlete groups than in the long-distance runner groups for the 180˚/s velocity. The total work of the plantar flexors for the control and triathlete groups was greater than the LDRG work at the 180˚/s velocity.

There were no significant differences among the groups in regards to the eccentric contraction of the plantar flexors at the 180˚/s velocity (Table 4).

DISCUSSION

This study compared the isokinetic dorsiflexor and ankle plantar flexor muscle strength and ground reaction force (kinetic analysis) of long-distance runners, triathletes and non-athlete individuals. In evaluating the first and second peak of the vertical force of the ground reaction, the long-distance runner group presented higher values than the

Table 2 - The means (SD) of the isokinetic variables at a velocity of 60°/s in the eccentric-concentric mode and concentric-eccentric mode and the comparison among the triathlete group, the long-distance runner group, and the control group.

	T Group	LDR Group	C Group	*p*-value
	Mean (SD)	Mean (SD)	Mean (SD)	
ECC/CO Mode				
PT ECC. FP (N.m)	35.81 (7.02)^{c}	35.99 (8.7)^{c}	40.57 (9.7)^{a,b}	0.008*
PT CON. DF (N.m)	33.35 (6.53)^{c}	33.53 (6.4)^{c}	37.33 (8.4)^{a,b}	0.009*
CO/ECC Mode				
PT CON. FP (N.m)	132.05 (25.02)^{c}	135.62 (24.22)	144.4 (27.13)^{b}	0.042*
PT ECC. DF (N.m)	136 (24.23)	135.04 (24.04)	145.48 (26.61)	0.072

T, triathletes; LDR, long-distance runners; C, controls; ECC, eccentric; CO, concentric; PT, peak of torque; PF, plantar flexion; DF, dorsiflexion; [a]significantly different from the LDR group; [b]significantly different from the T group; [c]significantly different from the C group; *$p<0.05$.

triathletes and controls. This difference may be explained by the regular training of the runners with weight bearing and the resulting functional changes, including a period of recovery and repair that requires less time than that of the triathletes, occurring even before new impact forces occur (1). When the platform used was not coupled to a treadmill, it was not possible to make all individuals use the same speed. However, the subjects were instructed to run on a 10.5-meter average path while training, and it was possible to calculate the average velocities (3.75 m/s $\pm$7%) of all of the participants. Thus, the higher values for the first and second peak may also be related to the running speed developed during training. This speed is higher and maintained for a longer duration in long-distance runners than in triathletes, as the amount of a triathlete's training hours per week is equivalent but with less time spent on lower-limb weight-bearing training, as their activities are not restricted to running. Furthermore, the footrace run by the triathletes has different muscle recruitment patterns than those associated with running alone, mainly due to the preceding cycling activity (20). The preceding activity promotes a reduction in speed and reduced impact force (21).

The isokinetic dynamometry of the control group participants made at 60°/s indicated a higher peak torque in the eccentric plantar flexor and concentric dorsiflexor activity of the two groups of athletes studied. The maximum torque of concentric plantar flexion was also greater in the control group than in the triathletes, but no significant difference was noted when comparing the control group and the runners. Peak torque is an indicator of muscle strength, and as isokinetic performance characteristics are capable of reproducing specific skills promoted by the demands of a sport, particular muscle results can be explained by distance running and triathlon training (22). The peculiarities of these two sports develop resistance to the detriment of the maximum force characteristics. Resistance exercise can cause a decrease in the cross-sectional area of muscle fiber, as well as an increased percentage of slow fibers and a decreased percentage of fast fibers (23). Thus, healthy, physically active non-athletes do not suffer this adaptation of specific strength training and may present higher maximum muscle strength than long-distance runners and triathletes during shear testing. The presentation of the best values of concentric plantar flexion peak torque by controls only compared with triathletes may be due to differences between long-distance running and triathlon running. Although both sports involve endurance, long-distance runners require more propulsive plantar flexion (24) and therefore can have a superior concentric muscle strength performance.

McCrory et al. (19) also used a velocity of 60°/s and measured the peak torque of the same ankle muscles of distance runners with and without Achilles tendinitis and found that healthy runners performed better. The authors suggest that a lack of power may represent a significant factor in repetitive stress injuries. Other authors have evaluated cross-sectional muscle area and indicated that strength deficiency may predispose military members and athletes to stress fractures (25). An adequate maximum strength value improves running economy (26), which triggers increased energy savings and the ability to absorb impact by the eccentric contraction of the plantar flexors when the foot lands on the ground (27). This effect can contribute to the prevention of tibia stress fractures.

The lack of difference when comparing the three groups with respect to the eccentric activity of the dorsiflexors and

Table 3 - The means (SD) of the isokinetic variables at a velocity of 180°/s in the eccentric-concentric mode and concentric-eccentric mode and the comparison between the triathlete group, the long-distance runner group, and the control group.

	T Group	LDR Group	C Group	*p*-value
	Mean (SD)	Mean (SD)	Mean (SD)	
ECC/CO Mode				
TW CO.DF (J)	70.65 (26.84)^{a}	65.17 (23.77)^{c}	93.88 (34.45)^{a,b}	0.000*
CO/ECC Mode				
TW CO.FP (J)	411.10 (115.98)^{a}	361.38 (72.68)^{b,c}	444.64 (153.49)^{a}	0.002*
TW ECC.DF (J)	211 (157.42)^{a}	127.21 (120.8)^{b,c}	229.44 (188.66)^{a}	0.004*

T, triathletes; LDR, long-distance runners; C, controls; ECC, eccentric; CO, concentric; DF, dorsiflexion; TW, total work; PF, plantar flexion [a]significantly different from the LDR group; [b]significantly different from the T group; [c]significantly different from the C group; *$p<0.05$.

Table 4 - The absolute and relative frequency of the eccentric contraction of the plantar flexors at the 180°/s velocity of the total sample and the comparison among the triathlete group (TG), long-distance runner group (LDRG), and control group.

	T Group	LDR Group	C Group	Total	*p*-value
	Frequency	Frequency	Frequency		
	n (%)	n (%)	n (%)	n (%)	
Contraction Development ECC. PF 180					0.081
Satisfactory	9 (34.6%)	4 (17.4%)	23 (44.2%)		
Unsatisfactory	17 (65.4%)	19 (82.6%)	29 (55.8%)		

T, triathletes; LDR, long-distance runners; C, controls; ECC, eccentric; PF, plantar flexor.

the relationship between plantar flexion and dorsiflexion in the concentric-eccentric mode during the 60°/s velocity may be associated with the intensity of the eccentric activity of the dorsiflexors during the race, which occurs after the final touch of the calcaneus and the support during the previous weight transfer and the flexion of the hallux (28). Thus, even at low speeds, such muscular activity can provide good performance without altering the relationship between concentric and eccentric plantar flexion-dorsiflexion.

At 180°/s, the triathlete and control groups exhibited higher total work values than the long-distance runner group in the eccentric and concentric dorsiflexion and concentric plantar flexion. When assessing the concentric contraction of the dorsiflexors, the control group presented higher values than the runners and triathletes. When determining total work, as determined after 30 repetitions at 180°/s, it is sensible to examine muscle fatigue (22). The results could suggest that long-distance runners have lower muscle strength and dorsiflexor-plantar flexor force than triathletes and non-athletes, but there is a decreased range of motion performed during the contraction, which is more evident in narrow running conditions. This reduction occurs through neuromuscular adaptations to ongoing training, resulting in greater ability to control the movement, which is characterized by a shorter duration of muscle activity and movement variability (29). This adaptation promotes increased speed during the race, as well as more rapid coactivation of antagonists before the end of the range of motion. Thus, as the total work measured by isokinetic dynamometry is given by the force multiplied by the distance across the range of motion, decreased amplitude can result in a reduction of the total work performed.

The control group performed the dorsiflexion-plantar flexion with greater amplitude during walking relative to the other groups, as these individuals are not adapted to a specific movement. The insertion angle of the heel during gait is 30.40, compared with 19.20 when racing (30). The greater angle of placement of the heel and the longer stance phase requires greater muscle action during walking. The specificity of physical training in triathletes has less influence due to the greater variability in their athletic movements (31). It is possible that the motor recruitment adaptions, which are different in each type of triathlon sport, do not promote a learning effect similar to what occurs with the runners (29). However, it is important to note that, in concentric dorsiflexion, the triathletes also exhibited lower values than the controls. This difference suggests that, even in the triathlon, the tendency of some athletes is to recruit before the concentric dorsiflexion phase of support and to run faster to concentric plantar flexion during landing with the front portion or the mid-lateral foot, reducing the time of the eccentric contraction of the plantar flexors, which often cannot even occur (25). This phenomenon might also explain the difficulty encountered by the groups of athletes during the testing of the eccentric plantar flexor at a 180°/s velocity, which was analyzed qualitatively and presented no statistically significant differences.

So et al. (32) observed that the total work at a speed of 180°/ s was significantly higher in a group of athletes than in non-athletic subjects, suggesting that regular training improves the resistance of the ankle, but evaluations of athletes practicing other sports (gymnastics, cycling and football), in which performance is not as dependent on the muscles of the ankle, does not generate specific amplitude adaptations. McCrory et al. (19) evaluated the total work (180°/s) in runners with and without Achilles tendonitis and found no significant differences between groups. An expected tendency toward lower values was observed in the injured athletes, which was likely due to pain, atrophy, reflex inhibition of muscular activity, and shortening.

Despite obtaining accurate isokinetic data on muscle performance, it was difficult to compare the current study with past studies due to differences in protocols, including differences in the number of repetitions, speed and type of contraction, as well as the individual and the brand positioning of the dynamometer. Furthermore, data are lacking in the literature on the isokinetic eccentric contraction of the ankle flexors and extensors at 180°/s, but for this study, the isokinetic dynamometer specifications required for performance were assessed using pilot studies to ensure the viability of the tests. As a 180°/s velocity approximates the typical athletic environment, it was important to assess this value in our study to assist in guiding the prevention of overuse injuries, such as stress fractures of the tibia.

Studies have shown that regular training programs that address the control of specific variables promote the improvement of the addressed aspects (33,34). Thus, data on the vertical forces of ground reaction and the performance of muscular strength and endurance of the ankle in distance runners and triathletes can assist in building training programs for the dorsiflexors and plantar flexors with specific goals (strength, endurance, and power at varying ranges of motion and speed). These goals can drive improvements in muscle condition during training and competition and thereby contribute to the prevention of overuse injuries, such as tibia stress fractures. In addition, these techniques can contribute to improved rehabilitation programs for these injuries. However, as these lesions are multifactorial, other studies that address the factors associated with other, intrinsic or extrinsic, factors must be conducted to properly evaluate these data in context.

The incidence of tibia stress fractures in runners and triathletes is partially due to a lack of public knowledge of such studies by professionals involved with work performance and rehabilitation. The biomechanics and muscle demands of isolated running have different characteristics than triathlon running, and therefore, the overhead mechanical factors change.

Thus, the analysis of kinetics and isokinetic differences between the two groups of athletes contributes to a better understanding of the biomechanical peculiarities of these sports and their possible relationship with the onset of tibia stress fracture. The study results will aid in the further study of specific variables with a goal of increased functionality for the type of sport addressed, as well as assisting in building rehabilitation protocols targeted toward tibia stress fracture with better delineated progression criteria.

In conclusion, the athlete groups presented lower muscle activity (strength and endurance) of the dorsiflexors and plantar flexors and higher values of the first and second peak ground reaction forces than the control group. The triathlete group presented lower values for these peaks and better endurance performance than the long-distance runner group.

AUTHOR CONTRIBUTIONS

Luna NM designed the study and participated in all the processes until the study was implemented. Alonso AC and Brech GC provided assistance in data collection and study design. Mochizuki L provided assistance in data analyses and manuscript writing. Nakano EY performed the statistical analyses. Greve JM guided the study.

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