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A study comparing gait and lower limb muscle activity during aquatic treadmill running with different water depth and land treadmill running

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Abstract

Aquatic treadmill running is a partial weight-bearing exercise for rehabilitation. The purpose of this study was to investigate the surface electromyography activities of the rectus femoris, tibialis anterior, biceps femoris and medial head of gastrocnemius, and gait kinematics during aquatic treadmill running in water levels at waist, mid-thigh and mid-shin and on land. Seventeen healthy subjects (9 males and 8 females) were recruited by convenience sampling. Participnts performed 2-min aquatic treadmill running at a specific speed for each water depth. The test speed was selected based upon the speed that elicited 110 steps per min. The surface electromyography data of lower limb muscles and the joint angles at three different water depths and on land were collected to evaluate the muscle activity and gait kinematics using a waterproofed surface electromyography system and inertial measurement unit for each muscle. Results showed that rectus femoris electromyography was different between depths during the swing and stance phases. Likewise, biceps femoris and tibialis anterior electromyography were different between depths for the swing phase. However, it was not the case for gastrocnemius electromyography. Peak flexion angles in both left and right hips were different between depths. A significant increase in a stance/swing ratio was observed with rising water depths. Water depth influenced muscle activity as well as kinematics. Aquatic treadmill running in the mid-thigh level should be further evaluated for its effectiveness, training value and applicability.

Keywords: aquatic treadmill running, muscle activity, motion analysis, electromyography.

Introduction

Running has been reported to have an association with high impact of loading (Macdermid et al., 2017). It could lead to the straining of joints in the lower limbs (Hamill et al., 1995), and contribute to a high rate of overuse injury (Daoud et al., 2012). Aquatic gait training (AGT) is widely recommended as an alternative to land based running for rehabilitation (National Library of Medicine, 2012). Primarily the value of an aquatic environment is the reduced load in terms of the supported weight bearing nature of the water. It has been suggested that partial weight-bearing (PWB) could relieve clinical symptoms in populations in respect to the upthrust force of buoyancy, reducing the weight bearing load in running in water relative to the volume of water displaced and the depth of immersion (Murray et al., 1993). Therefore aquatic treadmill running (ATR) not only has been included in managing post-operative clinical populations in both acute and chronic stages, but also in early rehabilitation for patients with muscle weakness, postural impairment and gait re-education (McCain et al., 2008).

Compared with changing the running environment from land to water, there were marked changes shown in gait biomechanics as a result of the influence of fluid mechanics on water locomotion (Harrison et al., 1992). It is believed that the properties of an aquatic environment such as buoyancy reduce the effect of gravity and thus promote body weight support. With a water immersion level of waist and mid-thighs, around 50% and 35% of body weight are supported (Koury, 1996) which could reduce compressive force, joint loadings and potentially optimize range of motion (ROM) of lower extremities while utilizing a full ROM is not possible or normally reduced on land. It could be illustrated precisely by a significant increase in ankle ROM and a decrease in hip ROM reported as the immersion level raises from the waist to the neck level and from the chest to the neck level (Jung et al., 2019). In addition to the spatio-temporal variables, longer stride length, shorter cadence, longer ground contact and swing time are also reported with increasing immersion depth (Macdermid et al., 2017).

Given the spatio-temporal changes reported, muscle activities could also be affected by the body immersion level during ATR. The frontal surface area of the limb immersed in water and velocity squared would directly affect the resistance to movement in the form of drag force acting in lower extremities. There may be a possible increase in muscle activation with an increase in water immersion as a result of a higher intensity of drag force (Sherman and Michaud, 1999). Knowledge of muscle activity during ATR is important since it has been suggested that this could potentially serve as a functional strengthening exercise (Jung et al., 2019).

ATR has been advocated for populations who failed to tolerate joint loadings brought by running (Macdermid et al., 2017). Despite the fact that the kinetic and kinematic characteristics of water running have been investigated in the last decade, many studies reported differences in muscle activation during ATR and land treadmill running (LTR) without manipulating water depth (Shono et al., 2007). To our knowledge, existing studies only compare gait variables in water and on land. Although a previous study has compared gait kinematics in various water depths (Jung et al., 2019), there has been no research on investigating the muscle activity during ATR at mid-thigh and mid-shin water depth. Therefore, the aim of this study was to compare the effects of different media (land and water) at different immersion depths (mid-shin, mid-thigh and waist level) on muscle activation and kinematics in ATR.

Methods

Participants

Seventeen healthy participants (8 females and 9 males) who were all recreational runners took part in the study. The demographic information of participants is summarised in Table 1. As inclusion criteria, participants needed to be able to walk without a walking aid and to be able to understand Cantonese or English instructions. Exclusion criteria included any history of musculoskeletal injuries or surgeries in the past 6 months, neurological disorders, cardiopulmonary conditions, or open wounds. Participants who were professional running athletes (International or Olympic group/ a minimum of 6 training sessions per week), with hydrophobia, and/or obesity were also excluded from the study. All participants were informed about the purposes, procedures and potential risks of the study before data collection. Then, the written informed consent forms were obtained from them. Before the experiment, participants were asked to fill in the Physical Activity Readiness Questionnaire (PARQ) to ensure their capability of participating in this research.

Design and Procedures

This was a cross-sectional study design. Participants were asked to perform 2-min running sessions using a motorized treadmill on land, and in three different water depths: 1) waist level (anterior superior iliac spine),

2) mid-thigh level, and 3) mid-shin level. The lateral view and the posterior view of running action were videotaped using an iPhone 11 at 30 frames/s to qualitatively view the gait. The recording devices were placed 1 m laterally and 10 m posteriorly away from participants, positioned at the knee crease level to prevent angulation of the video. The muscle activities of the rectus femoris, tibialis anterior, biceps femoris, and medial gastrocnemius of both legs during running were recorded using a 16-channel sEMG system (aktos surface EMG transmitters, Myon, Switzerland) and a customized data logger at a 2000 Hz sampling rate. The sEMG signals were exported using EMGandMotionTools Version 6.0.4.0 (Cometa, Milan, Italy). The sagittal joint angles were recorded using the Inertial measurement unit (IMU) system (aktos-t 3-axial sensors, Myon, Switzerland) with a sampling rate of 286 Hz and a proprietary bidirectional 2.4 GHz transmission protocol.

Data collection procedures began with the measurement of anthropometric data as follows: (1) body weight using an electronic scale (BC-730b, Tanita, Japan), (2) body height using a measuring tape, and (3) blood pressure using a sphygmomanometer (omron HEM-7211). The BMI was determined through the calculation of participants' height and weight.

After the anthropometric measurements, the electrodes were attached to the skin of each individual to record the rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA) and medial gastrocnemius (mGas) muscle activity according the SENIAM recommendations (Hermens et al., 2000) after standard skin preparation procedures by shaving the hair around and using alcohol swab to reduce skin impedance. These muscles were selected based on their role in the gait. Waterproof dressing 3M™ Tegaderm™ Transparent Film Roll 16004 was applied over the electrodes to reduce the influence of water on EMG signals and for waterproof protection for the electrodes (Carvalho et al., 2010).

Then, each participant performed the muscle isometric maximal voluntary contraction (MVC) for subsequent EMG data normalization. During the MVC task, participants increased the force gradually till reaching the maximum effort after 3-5 s, then held it for 3 s and relaxed for another 3 s. Two repetitions of MVC trials were performed with a 60-s rest interval between the two consecutive trials. MVC testing procedures are listed in Table 2.

Afterwards, the inertial measurement unit (IMU) sensors were attached to the participant's body (mid-thigh, mid-shin, and dorsum of feet) according to the altos-t system and covered by the same waterproof dressing, followed by IMU calibration.

Participants were asked to enter the motor driven treadmill (LIFESTYLE The Ultimate Hydrotherapy System, HYDRO PHYSIO®□) after being instrumented with sEMG and IMUs. Each participant underwent an adaptation period in order to get used to the equipment and data collection. All participants had no prior experience with ATR. Subsequently, a 1-min practice trial with 110 bpm by a metronome app (soundbrenner) was used during both land treadmill testing and aquatic treadmill testing at the waist level to familiarise participants with running (Jung et al., 2019). No horizontal water flow or artificial current was used during ATR.

In the experiment, participants completed 2-min test trials, with hands off the rails, on land, and aquatic treadmill adjusted to three water levels (with participants in standing): waist (ASIS), mid-thigh and mid-shin level. The test speed was selected based upon the speed that elicited 110 steps per min paced by the same metronome app. Meanwhile during running, sEMG and gait kinematics were collected and analysed. A 5-min rest interval was provided after each test trial to minimize fatigue.

The Borg rating of perceived exertion (RPE) (0-10 scale) (Health, 1998) was measured every minute to record the effort and the level of fatigue during the study.

Statistical Analysis

Raw EMG signals were processed with a bandpass filter (cutoff frequencies between 20 and 300 Hz) and a root-mean-square sliding window (50 ms time constant) (MatLab2017a; Mathematical computing software, Natick, MA, USA), with the left peak hip flexion angle used to divide the period of each gait cycle. After initial 10 gait cycles, amplitude of EMG signals for the muscles from 10 gait cycles was selected and averaged. Similarly, raw MVC data were filtered and smoothed with the same method. The largest mean MVC value during contraction was selected as the MVC value for each muscle. Then, mean sEMG data for the 10 gait cycles were normalised to these MVC values and expressed as %MVC. sEMG activities during the stance phase and the swing phase were analysed separately. As for kinematic data, the peak hip flexion angle was analysed using IMU to indicate initial contact as the start of the stance phase. Stance and swing phases were differentiated using the peak hip flexion angle and the peak hip extension angle for MVC data as well.

Muscle activity and kinematic statistical calculations were computed using Matlab 2017a and SPSS 21, respectively. Descriptive data were calculated for demographic data (age, body height, body weight and BMI) as well, moreover, %MVC for each muscle per water depth condition was calculated. The data are expressed as means ± standard deviations. Total mean of sEMG activities in terms of %MVC was analyzed using a 2 (leg side: left and right) x 2 (task type: stance and swing) x 4 (muscle type: RF, BF, TA and mGas) x 4 (treadmill running condition: land, mid-shin, mid-thigh and waist level) repeated measures ANOVA. The alpha level was set at 0.05 for all statistical computation. Post-hoc comparisons with least significant difference procedures were used to determine any significant differences between the specific levels of the factors.

For gait kinematics, one-way ANOVA was used to compare the peak angle of different joints and the swing/stance ratio across different water depths. The alpha level was set at 0.05 for all statistical computation. Post-hoc comparisons with Fisher's least significant differences were used to identify any significant interactions and differences between the specific levels of water depths.

Results

Muscle activity response

%MVC of RF, TA, BF and mGas during different phases are presented in Table 3. The results of repeated measures ANOVA showed that the muscle type ($F(3, 57) = 8.607$, $p < 0.001$), task type ($F(1, 19) = 18.151$, $p < 0.001$), and treadmill running condition ($F(3, 57) = 8.080$, $p < 0.001$) were significant factors that influenced %MVC. However, the leg side was not the case. Also, there was a significant interaction between the muscle type and the task type ($F(3, 57) = 28.320$, $p < 0.001$) in %MVC.

In the stance phase, for both right and left sides, there was no significant difference in %MVC of RF, TA and mGas across the land and three different water depths in spite of an increasing trend of muscle activity with an increasing water level ($p > 0.05$). The difference in %MVC of BF was significant across the land and three different water depths for the left side ($p = 0.008$), but not significant for the right side ($p = 0.085$). As reflected from the post-hoc test, significant differences were present when comparing land and mid-shin ($p = 0.015$), mid-thigh ($p = 0.018$) and waist ($p = 0.011$) levels, respectively.

During the swing phase, both right and left sides, RF showed a significant difference in %MVC between water and land conditions ($p < 0.05$). Post-hoc testing revealed significant differences on the right side when comparing land and mid-shin levels ($p < 0.001$), the mid-thigh level ($p = 0.001$), and the waist level ($p = 0.003$), also between mid-thigh and mid-shin ($p = 0.050$), and waist ($p = 0.030$) levels. Meanwhile, on the left side, statistically significant differences could be found when comparing land and mid-thigh levels ($p = 0.020$), land and waist ($p = 0.006$), mid-shin and mid-thigh ($p = 0.003$) levels. These showed an increasing trend in muscle activities until the mid-thigh level, but dropped in the waist level in both legs. The muscle activity of RF during the swing phase in all water levels was higher than the land level. For TA, there was no significant difference observed on the right side ($p = 0.319$) in terms of %MVC across four conditions. However, statistically significant differences could be seen on the left side ($p = 0.001$) with the post-hoc differences revealed when comparing land and mid-shin ($p < 0.001$), mid-thigh ($p < 0.001$) and waist ($p = 0.002$) levels. For BF, the difference of %MVC was not significant on the right side ($p = 0.074$), but significant on the left side ($p = 0.012$). Post-hoc tests for the left side revealed significant differences between land and mid-shin ($p = 0.021$), land and waist ($p = 0.021$), mid-shin and mid-thigh ($p = 0.030$), mid-thigh and waist ($p = 0.36$) levels. For mGas, there was no significant difference observed on the right and left sides in terms of %MVC across four conditions ($p > 0.05$).

Peak angle

One-way ANOVA was used to compare the peak hip, knee and ankle angles across different water depths for the entire stride and the results are presented in Table 4.

A statistically significant difference was found in the left hip peak angle among the land and three different water depths ($p = 0.033$). Significant differences were found in all four comparisons between land and the mid-shin level ($p = 0.032$), land and the mid-thigh level ($p = 0.036$), mid-shin and waist levels ($p = 0.034$) and between the mid-thigh and waist levels ($p = 0.038$) using post-hoc comparisons. However, no significant difference was found when comparing land and waist conditions, and when comparing mid-shin and mid-thigh conditions. Generally, it showed a trend of increasing the peak angle of hip flexion from land to the thigh level, but decreased in the waist level.

Similarly, a statistically significant difference was shown in the right hip peak angle when comparing land and three different water depths ($p = 0.049$). A significant difference was found between mid-shin and waist levels ($p = 0.049$), yet no significant difference was found in comparisons of peak angles in other water levels.

As for the peak knee angle, no statistically significant difference was shown in both the left ($p =.595$) and the right leg ($p = 0.481$). Also for the peak ankle angle, no statistically significant difference was found for the left ($p = 0.633$) and the right leg ($p = 0.335$).

Swing/stance ratio

One-way ANOVA was used to compare the stance/swing ratio across land and different water depths and statistical analysis is presented in Table 5. There was a significant increase in the swing/stance ratio across land and different water depths (both sides: $p = .000$) on both sides. Post-hoc analysis revealed that on the left side, there was a significant increase in the swing/stance ratio when comparing land and mid-thigh ($p = .006$), land and waist ($p = .000$), mid-shin and waist ($p = .000$), also mid-thigh and waist ($p = .009$) levels. As for the right side, post-hoc analysis showed significant differences between land and waist ($p = .000$), mid-shin and waist ($p =.000$) as well as mid-thigh and waist levels ($p =.047$). The trend reflected an increase in the ratio with rising water depths.

Borg rating of perceived exertion (RPE)

The RPE values between land treadmill and aquatic treadmill running at different depths showed a significant difference between groups ($p = 0.017$). In subgroup analysis, land running compared with shin immersion aquatic treadmill running and waist immersion aquatic treadmill running showed significant changes ($p = 0.003$ and $p = 0.015$, respectively).

Discussion

Relationship of muscle activity and peak joint angles across water levels

This study indicates that ATR significantly increased selected muscle activities at different levels of water immersion when compared to land treadmill running. Immersion depth is an important component of hydrotherapy. It is clinically relevant to examine the effect of water depth on the gait. Previous studies have discussed effects of immersion depth on either muscle activities or kinematics, without correlating both. A lack of evidence for the optimum water depth with consideration of muscle activation and kinematics underwater makes clinical decision difficult. Acknowledging the relationship between immersion depths, muscle activation and the gait pattern would help identify potential benefits and provide useful guidelines to make clinical decisions for those using ATR.

Additionally, different water depths during ATR could influence many aspects of muscle activity. For example, there was an increase in muscle activity of the RF till the mid-thigh level, but a decrease in the waist level. This can be explained by an increase in water depth, a longer period of the swing phase and acceleration of an added water mass when performing ATR (Silver et al., 2014). This contributes to an increase in muscle activities in the RF as more hip flexion range is required to work against the effects of hydrodynamic resistance in the swing phase (Silvers et al., 2008). Notably in the mid-thigh level, in order to reach an economical locomotion, effects of hydrodynamic resistance are reduced with greater hip joint flexion range, inducing more knee flexion range (Kato et al., 2001). This also explains why the largest peak hip flexion angle was observed in the mid-thigh level. Our results also show the largest peak knee angle in the mid-thigh level, albeit non-significant. This is consistent with a previous study showing no significant difference in the peak knee angle due to a smaller and streamlined surface area of the shank (Jung et al., 2019). Moreover, as half of the leg is immersed in water, the RF is responsible for overcoming the skin friction drag while lifting the leg above the water surface and the hydrodynamic resistance to perform hip flexion, in addition to knee extension. This leads to the highest %MVC of the RF in the mid-thigh level. However, the effect of buoyancy offsets the effect of hydrodynamic resistance in te waist level, giving rise to more body weight support and thus less recruitment of the RF to flex the hip during the swing phase. Also, as the limbs (i.e., lower extremity) were fully immersed while running, overall muscle activity of the RF decreases.

Importantly, our results confirm that both the BF (stance) and TA (swing) show an increasing muscle activity trend with increasing water depth which is coherent with other studies. The BF is a biarticular muscle which can perform hip extension and knee flexion. Silvers et al. (2014) observed that the BF during walking after heel contact acts as hip extensors together with the gluteus maximus to perform hip extension. Takeru et al. (2002) also reported the BF's role as hip extension instead of vertical support during the stance phase. Since hip extension is needed when transitioning from heel strike to the pre-swing phase, therefore this can explain our results as higher BF activity is needed to counteract stronger drag force with an increasing water level.

These results are in contrast with the significant decrease in muscle activity suggested by Liebenberg et al. (2011). It was reported that reduction in effective body weight (i.e., increased body weight support) resulted in a reduction in muscle activity (Liebenberg et al., 2011). However, it is important to note that in that study, a lower body positive pressure treadmill was used to provide body weight support and the study did not address the effect of hydrodynamic resistance as it only provided body support to imitate the buoyancy effect provided in the water environment. Therefore, a combination of hydrodynamic resistance and buoyancy may affect the pattern of muscle activities.

For the muscle activity outcome observed in the TA, our results showed that the muscle activity increased with increasing water depth. It is partly consistent with the study showing a significant difference between land and waist level running at 0.8 $m \cdot s^{-1}$ (Takeru et al., 2002). This pattern could be explained by the role of the TA to overcome more hydrodynamic resistance to dorsiflex the ankle in preparation for foot strike during the swing phase (Kato et al., 2001). Also, it is supported by the evidence of an increased swing/stance ratio which may prolong the muscle activation of the TA in the swing phase. However, our results are inconsistent with Silvers et al. (2014), showing no significant difference when comparing ATR and LTR.

Gait kinematics in different water levels

Overall decrease in hip ROM in the waist level

There was a decrease in overall hip range of motion in the waist level compared to the other three conditions. It could be the result of increased resistance and buoyancy due to increased water depth (Jung et al., 2019). More resistance makes weight transfer over the standing foot more difficult. Besides, more up-thrust force due to increased buoyancy may also interfere with weight transfer. Therefore, a decrease in hip extension occurs during the mid to late stance phase, reducing the hip ROM.

Swing/stance ratio

In our study, an increasing swing/stance ratio across different water depths was observed. Our findings support the qualitative results of other studies. When performing ATR at the waist level, more body support can be provided with buoyancy which can subsequently influence the flight phase during running. This enhances the dynamic balance, enabling participants to maintain balance even with longer single-leg stance time (Baezner et al., 2008). In addition, since fewer body parts are affected by lower air drag force with increasing water depth, hydrodynamic resistance in the form of drag is also higher (Machado, 2010). Hydrostatic resistance, caused by limb movement speed and the limb frontal surface area, may decrease velocities during limb movement (Macdermid et al., 2017). When combining the effects of buoyancy and drag forces during ATR at a constant speed, it apparently prolongs the swing phase duration (Silvers et al., 2014). This is in line with the qualitative findings showing the delay pattern shown in ATR at the waist level, meaning that the peak angle of joints at the waist level usually comes after other levels.

Although the conditions varied greatly, it is meaningful to note that there were many similarities in muscle activity and kinematics between conditions. Humans tend to have a preferred gait pattern. It is natural for humans to accommodate their gait to compensate variations in human postures, and to adapt a natural gait pattern under different conditions for equilibrium and stability (Carlos et al., 2017). It could be due to the fact that one of our limitations of this study was the under control speed. The control of speed could have constrained participants in using a narrow range of gait patterns. Subsequent research should be conducted which would allow participants to select a preferred speed at each water depth. Likewise, our understanding of the influence of water depth on muscle activity would be enhanced by having participants exercise at the same exercise intensity for each depth. The importance of the present study is that we demonstrated muscle activity at different depths when speed of locomotion was controlled.

Clinical implications

ATR is potentially applicable in rehabilitation for patients with anterior cruciate ligament (ACL) reconstruction as the results show a significant increase in RF and BF muscle activities compared to the land condition. In order to determine whether recovery is achieved, muscle strength of the quadriceps and hamstrings is an important criterion to be considered for dynamic stability of the knee joint. Yet, as suggested by several protocols, knee flexion can only be resumed at least three weeks after surgery, contributing to quadriceps weakness (Wilk et al., 2012). Thus, ATR may be considered as an early intervention to strengthen both muscles in a less stressed environment, which provides an increased body weight support to the reconstructed ACL while decreasing the joint loading and reducing the chance of reinjury.

Possibilities of using ATR for balance training in elderly can also be explored. Hip strategy is used more often in elderly for balance (Woollacott and Manchester, 1993) as hamstrings are activated to a greater extent to stabilise the hip joint (Benjuya et al., 2004). According to our findings, activity of hamstrings increases in ATR, which can potentially become a balance exercise for the elderly.

From our study, significant findings can be seen in muscle activity at the mid thigh level, thus future studies considering different speeds can be conducted. Also, further investigation on physiological differences will help obtain comprehensive understanding of gait mechanics and economy in response to various water depths, with more exploration on the associated benefits of depth in relation to musculoskeletal injury. It may be valuable to look into muscle patterns during ATR as well, such as magnitude and coordination of muscles at different angles, which can be filtered for the general muscle pattern overtime.

Limitations

Despite the implications, this study has few limitations. The sample size was relatively small, consisting of only 17 participants. This in turns limits the study power, which could also explain the inconsistent finding between two legs. Another limitation is the heterogeneity in the participants' characteristics. The age range was narrow and this study evaluated healthy participants, limiting its external validity as well as application to patients with musculoskeletal disorders. Some other confounding variables include morphology issues, limb length discrepancy, mass distributed between both sides and dominance of limbs, which may need to be addressed in future study.

Conclusion

In conclusion, our study showed a significant difference in muscle activities and gait kinematics across different water depths in healthy participants running at the same speed, which has significant implications for rehabilitation. We observed that the rectus femoris had the highest muscle activation during the swing phase in the mid-thigh level. Meanwhile, the biceps femoris (stance) and the tibialis anterior (swing) had an increasing muscle activity trend with increasing water depth, which is in line with other studies. An overall decrease in hip ROM with a prolonged swing phase was observed in the waist level.

It was noted that hip ROM was under the influence of water depths. Interaction between hydrodynamic resistance and buoyancy as a result of change in water depth was the main factor for the gait variables and muscle activity changes. Medical professionals should be aware that water depths may impact the treatment results when making clinical decisions in considering the level of water immersion in exercise prescription. Based on our findings, ATR in the mid-thigh level should be further evaluated for its effectiveness, training value and applicability in an ACL rehabilitation program.

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Table 1.

Descriptive characteristics of the seventeen participants.

Demographic Factors	Minimum	Maximum	Mean	SD
Age (year)	19	21	20.71	0.59
Height (cm)	153	178	167.42	0.07
Weight (kg)	44.5	70.4	56.80	6.61
Body mass index (kg/m^2)	18.14	25.86	20.23	1.71

Note: SD = Standard deviation.

Table 2.

Locations of the electrodes.

Muscle	Location of the electrode (both left and right lower limbs)	MVC testing procedures
RF	50% on the line from the anterior spina iliaca superior to the superior part of the patella	Participants are positioned in sitting, knee in 90 degree flexion. Maximal resistance is given on the anterior distal end of the lower leg pushing into flexion while the participant is instructed to maintain 90 degree knee flexion. Thigh is stabilised while participants support themselves on the plinth.
BF	50% on the line between the ischial tuberosity and the lateral epicondyle of the tibia	Participants are positioned in prone, knee in 90 degree flexion. Maximal resistance is given proximal to the ankle joint on the posterior aspect of the lower leg pushing into extension while the participant is instructed to maintain 90 degree knee flexion. Thigh is stabilised.
TA	1/3 on the line between the tip of the fibula and the tip of the medial malleolus.	Participants are positioned in supine, ankle in the neutral position. Maximal resistance is given on the medial dorsum of the foot pushing into plantarflexion and ankle eversion while the participant is instructed to maintain the ankle in the neutral position. Lower leg is stabilised.
mGas	Most prominent bulge of the muscle	Participants stand in tip-toe. Maximal resistance is given on the shoulders pushing participants back to the normal standing position while the participant is instructed to maintain the tip-toe standing position.

Note: BF = biceps femoris; mGas = medial head of gastrocnemius; MVC = maximal voluntary contraction; RF = rectus femoris; TA = tibialis anterior.

Table 3.

Mean, standard deviation of %MVC of RF, TA, BF and mGas muscle at different water depths during different phases

Left				Right			
Muscle	**Water depth**	**Stance**	**Swing**	**Muscle**	**Water depth**	**Stance**	**Swing**
RF	Land	4.46 ± 3.10	8.23 ± 19.18	RF	Land	4.91 ± 3.88	3.94 ± 3.60
	Mid-shin	5.40 ± 2.93	15.88 ± 11.11		Mid-shin	5.85 ± 5.28	14.65 ± 9.77
	Mid-thigh	8.80 ± 6.37	21.90 ± 11.36		Mid-thigh	8.63 ± 8.77	21.71 ± 17.76
	Waist	11.64 ± 16.24	19.26 ± 19.74		Waist	8.91 ± 13.25	13.35 ± 10.29
TA	Land	11.28 ± 6.80	12.81 ± 7.81	TA	Land	13.19 ± 8.45	14.48 ± 10.70
	Mid-shin	17.93 ± 15.91	23.53 ± 11.27		Mid-shin	13.50 ± 7.95	17.65 ± 7.87
	Mid-thigh	16.75 ± 14.39	26.98 ± 22.24		Mid-thigh	12.17 ± 9.28	20.11 ± 14.35
	Waist	17.18 ± 8.54	27.87 ± 18.71		Waist	11.01 ± 6.46	18.03 ± 15.38
BF	Land	10.94 ± 9.46	6.95 ± 5.98	BF	Land	11.45 ± 10.58	5.90 ± 4.76
	Mid-shin	15.60 ± 11.74	3.38 ± 1.89		Mid-shin	15.89 ± 11.21	4.30 ± 3.97
	Mid-thigh	17.23 ± 15.96	6.15 ± 5.71		Mid-thigh	17.43 ± 10.52	6.88 ± 4.64
	Waist	17.55 ± 15.63	3.50 ± 1.70		Waist	17.14 ± 11.84	4.44 ± 3.36
mGas	Land	30.09 ± 34.76	5.60 ± 6.94	mGas	Land	39.10 ± 25.67	4.61 ± 2.97
	Mid-shin	37.30 ± 36.10	7.82 ± 11.82		Mid-shin	35.07 ± 22.08	9.05 ± 11.90
	Mid-thigh	36.02 ± 33.15	12.85 ± 17.49		Mid-thigh	35.82 ± 27.86	14.48 ± 24.34
	Waist	32.69 ± 42.70	12.70 ± 17.10		Waist	34.97 ± 43.54	15.62 ± 26.78

Note: BF = biceps femoris; mGas = medial head of gastrocnemius; MVC = maximal voluntary contraction; RF = rectus femoris; TA = tibialis anterior.

Table 4.

Mean of peak angles of the hip, knee and ankle at different water depths.

Left			**Right**		
Joint	**Water depth**	**Mean of peak angles**	**Joint**	**Water depth**	**Mean of peak angles**
Hip	Land	35.74	Hip	Land	35.04
	Mid-shin	34.65		Mid-shin	34.89
	Mid-thigh	40.15		Mid-thigh	37.73
	Waist	35.19		Waist	32.35
Knee	Land	75.46	Knee	Land	75.41
	Mid-shin	76.78		Mid-shin	76.18
	Mid-thigh	79.06		Mid-thigh	76.35
	Waist	74.96		Waist	71.35
Ankle	Land	11.26	Ankle	Land	12.95
	Mid-shin	10.65		Mid-shin	7.68
	Mid-thigh	13.09		Mid-thigh	10.61
	Waist	16.41		Waist	16.19

Table 5.

Statistical Analysis of the stance/swing ratio at different water depths

	Stance/swing ratio					Pairwise comparison (P value)					
	SS	df	Mean Square	F	*p*	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4
L	0.48	3	0.16	11.68	**.000**	.265	**.006**	**.000**	.091	**.000**	**.009**
R	0.14	3	0.05	8.82	**.000**	.973	.205	**.000**	.414	.000	**.047**

Note: L = left; R = right; BF = biceps femoris; mGas = medial head of gastrocnemius; RF = rectus femoris; TA = tibialis anterior; SS = sum of squares; 1 = on land; 2 = on the mid-shin level; 3 = on the mid-thigh level; 4 = on the waist level; bold numbers indicate significance at $p < 0.05$.