Effects of Cooling on Ankle Muscle Maximum Performances, Gait Ground Reaction Forces and Electromyography (EMG)

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EFFECTS OF COOLING ON ANKLE MUSCLE MAXIMUM PERFORMANCES, GAIT GROUND REACTION FORCES AND ELECTROMYOGRAPHY (EMG)

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Dedication

To

My heavenly ‘Father’.

And

My beloved ‘Mother’**.**

Declaration

I certify to the best of my knowledge and belief that this thesis does not:

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(ii) Contain any material previously published or written by another person except where due reference is made in the text; or
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Abbreviations

ATP- Adenosine tri-phosphate, molecular unit of intracellular energy transfer.

µV - Micro volt, the unit of electrical amplitude.

DF - Dorsal or dorsiflexion, Foot movement in upward direction.

EMG - Electromyography.

FG - Fast glycolytic.

FOG - Fast oxidative glycolytic.

GM - Gastrocnemius Medialis Muscle.

GRF - Ground Reaction Force.

HS - Heel-Strike.

Hz- Hertz, the unit of frequency.

Kg - Kilogram, the unit of mass.

MPF - Mean Power Frequency.

MVC - Maximum voluntary contraction.

Norm - Normalized.

N- Newton, the unit of force.

Ohm- The unit of electrical resistance.

PF - Plantar flexion, Foot movement in downward direction.

RCOF - Required Coefficient of Friction.

RMS- Root Mean Square.

RH - Relative Humidity.

SD - Standard Deviation.

SS-Single Step.

SENIAM -Surface Electromyography for the Non-invasive Assessment of Muscles.

TA - Tibialis Anterior Muscle.

TO - Toe-Off.

Tsk - Skin Temperature.

Vs. - Versus.

Abstract

Background: Temperature is considered as a significant determinant of skeletal muscle function and also a predisposing factor for muscular mechanical performance. Yet, the effects of local cooling on neuromuscular function and performance during gait remain unexamined.

Objectives: To investigate the effects of local cooling on lower leg muscles' isometric maximal force production, electromyography (EMG) and gait ground reaction force.

Methods: Experimental study, within subject design**.** Sixteen healthy university students, age (mean ± SD) 27.0±2.9 years participated in the study. The local cooling was induced by immersing both lower legs up to knee for 20 min in cold water (10°C) in a climate chamber. Electromyographic (EMG) activities and the ankle dorsi and plantar flexors maximum isometric forces were measured in tibialis anterior and gastrocnemius medialis muscle by using surface electrodes and dynamometer. Ground reaction forces during gait were measured on a walkway with force plate.

Results: There was a significantly reduced isometric maximum force in tibialis anterior (TA) muscle (P<.001) after cooling. The mean EMG amplitude of gastrocnemius medialis (GM) muscle was significantly increased after cooling (P<.003). There were no significant changes in ground reaction forces and RCOF in gait trials after 20 min cooling.

Conclusion: Neuromuscular performances were partly altered after cooling. Maximum strength loss occurred in dorsi-flexion. Fatigued, over-exerted power loss was observed in EMG during plantar flexion. These muscular changes did not contribute significantly to normal gait ground reaction forces on dry and level surface. These may indicate that 20 min cooling in the cold water at 10°C can influence our maximum muscle performance, but the cooling may not be strong enough to impact our daily general and sub maximal activities.

Keywords

Peripheral cooling, Ground reaction force, Required coefficient of friction, Gait, Muscle maximum performance, EMG.

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1. Introduction

1.1. Background of Muscle Cooling and Performance

Temperature is considered as a significant determinant of the contractile and metabolic properties of skeletal muscles [1, 2] and also a predisposing factor for muscular mechanical performance [3]. Muscle activities such as force generation, contraction, relaxation and power output vary with the body temperature changes. Maximal loco-motor speed and reaction rates may change with temperature and be slow in the cold [4]. Generally, it is known that both hypothermia and hyperthermia may impair muscle function; it is evident that there must be an optimal temperature range where the best performance of the muscle occurs. The temperature of the muscle needs to be a bit higher in a thermo-neutral environment in order to get the maximum short-term power from the major limbs muscles. [5]. Now-a-days, it is essential to know more detail about the temperature effect on the muscle force development and relaxation in human muscle because changes in temperature may constrain the performance [1]. Temperature is also considered as intense effecter on the neuromuscular transmission system of both afferent and efferent impulse which may be slowed due to cooling of the nerves [6]. However, controversial evidence is found in the literature which has examined the effects of decreased muscle temperature on muscle contractile properties. There have been several reports that muscle contraction force and rate of force development were impaired at low muscle temperature [7-10].

Fatigue is a general concept that is generally defined as a temporary loss in force generating capacity [11] or an acute impairment in performance due to previous muscle contractions leads to the incapable of necessary force production, that is, volitional exhaustion [12,13]. Homeostasis is being maintained by the physiological regulatory control processes occurring throughout the body [2]. Sometime it is not possible to maintain homeostasis through the physiological mechanism to keep limb muscle temperature close to the optimal range even in moderately cold conditions. Theory of the regulatory systems maintaining homeostasis is that of thermoregulation. And within the homeostatic range human body core temperature

is relatively well protected even skin and peripheral muscle temperature may change under different severe temperature exposures. [5].These can be used as a model to investigate the regulatory processes that influence physical performance, the development of fatigue [2]. Rapid onset of fatigue due to greater activation of fast twitch muscle fibers and rate of recovery from fatigue also appears which allows prolong endurance of muscle activity [14, 15]. In spite of, some muscle properties, way of cooling, fiber and type of contractions there is consensus. Muscular functional properties rate slowed down with almost any degree of cooling therefore considerably reducing the muscle force. Reduced force production due to slowed nerve conduction velocity and enzymatic processes responsible for lowering local muscular endurance during dynamic muscular contraction. In below 27°C both the voluntary muscular contraction and inducing force progression capabilities are started to affect. Most of these effects occur without disturbance of central activation or core cooling. [2]. It is possible when aware about cold weather exposure and thus insulate body wearing extra inner layers of cloths to maintain the central core temperature but keep their limbs exposed [16].

A large extent of studies investigating cooling effect on human performance involves core cooling. Many performance effects related to the peripheral cooling, method yet-to explore the effects of cooling when muscle cooling temperature range varies from (10°-42°C) [2].The studies looked over muscle fatigue under isometric conditions at different temperatures by voluntary sub-maximal sustained contractions where the certainty of the effect of temperature was not ensured. De Ruiter et al. [1] investigated the effect of temperature on the rates of isometric force development by introducing different temperatures of water baths 37°, 31°, 25° and 22°C immersed human lower arm for 20 min in water baths where isometric force became reduced below 25°C. Though, water temperatures from 32°-22°C decreased muscle fatigue parameters related with muscular force production. Contemporarily, rates of muscle force have been shown to decrease at low temperatures in numerous animal studies [1]. Human experienced exceeding the muscle distal temperature on the effects of environmental cooling without developing the core temperature within a strict homeostatic range [7, 17]. Adjustment of skeletal muscle functions with changes of ambient temperature such as irregular uncomfortable cold and heat that affects mechanical contractile properties of muscle. Contractile properties are important to understand the

effects of cooling or heating on performance such as muscular strength, power, and endurance [2, 7]. Lower limbs are very difficult to guard from cooling in extreme cold weather, except cold-protective booths [18]. Peripheral muscle exposed to a large thermal variation as much as 10 degrees [7] from high 41°C and low 21°C depending upon the level activity, clothing, time and environment throughout the day [19]. Since 1868, many studies have focused on the muscle function, performances influence on temperature [3]. Immersion of limb into cold water is a relatively high rate of heat loss compared to air cooling, although there are circumstances in which a degree of cold exposure may affect power generation in sports on land [5]. Previous studies scrutinized work capacity in extreme cold environments concentrated to the systemic effects of cooling to survive in many occasions and accidental cold water immersion. However, now a-days, long exposure of cold occurring in professional, sports, and recreation activities increases, exploring about the capacity to perform work and exercise in cold environments therefore, is essential. Some sports may require long stay in cold or wet weather and there is a chance of central hypothermia. In these circumstances, it is difficult to avoid peripheral cooling meanwhile keeping the body's core temperature normal or even hyperthermic. [2].

Various studies have proved that cooling may hinder muscular performance [20] as a result of biochemical changes e.g. declined ATP-hydrolysis or availability and calcium release and uptake impaired in the muscle from the sarcoplasm due to impairment of sarcoplasmic reticulum ATPase. Calcium assists the release of acetylcholine from the synaptic vesicles in the motor neuron terminal and to control the position of the regulatory proteins troponin and tropomyosin. The slowing of calcium reuptake thus leads force production without required stimulation. [14,21]. Enzymatic activity, rate processes such as time to twitch or voluntary contraction or relaxation of muscle contraction and rate of onset of fatigue has been studied with the advance of science. Through studying these properties and using these instruments may help us to be aware of the effects of local muscle cooling on human performance. [2]. There were no such studies found who assessed the effect of cooling on the electrical activation and force generating capacity of a previously fatigued muscle. Only very recent study by Cè et al. [22] showed after fatigue, muscle cooling decreased conduction velocity in low temperature. There was an optimal temperature for each individual above or below which the endurance of isometric contraction dropped remarkably

[23]. Evidence from previous human research that muscle cooling in water at 10°–12°C and intramuscular temperature 28°C, significantly increased the glycolysis from glycogen in both the red and white muscles, leading to a rapid accumulation of lactate during exercise [24]. Because the local vasoconstriction in tissues exposed to cold is likely to reduce the oxygen supply, modifying the metabolic pathways at the same time, muscle cooling also decreased the oxygen extraction and impaired the oxidative reactions [25]. Through high intensity dynamic frequent work developed fatigue earlier when muscle temperature is lowered [26]. Abbiss et al. [27] in their study compared fatigue in hot and cold temperature where they set 10°C for the cold environment to get the measurements of power output, rectal and skin temperature, activation of different muscle groups of lower limbs, ratings of perceived exertion, thermal sensation and pain intensity. The results showed that power output declined during exercise in the heat rather than in cold [27]. When considering the effect of temperature on muscle function, power output and fatigue, it is necessary to look into the past effects of temperature on the contractile process. Limb cooling might affect power production at one or more of the several steps from volition to power output itself (See Figure 1).

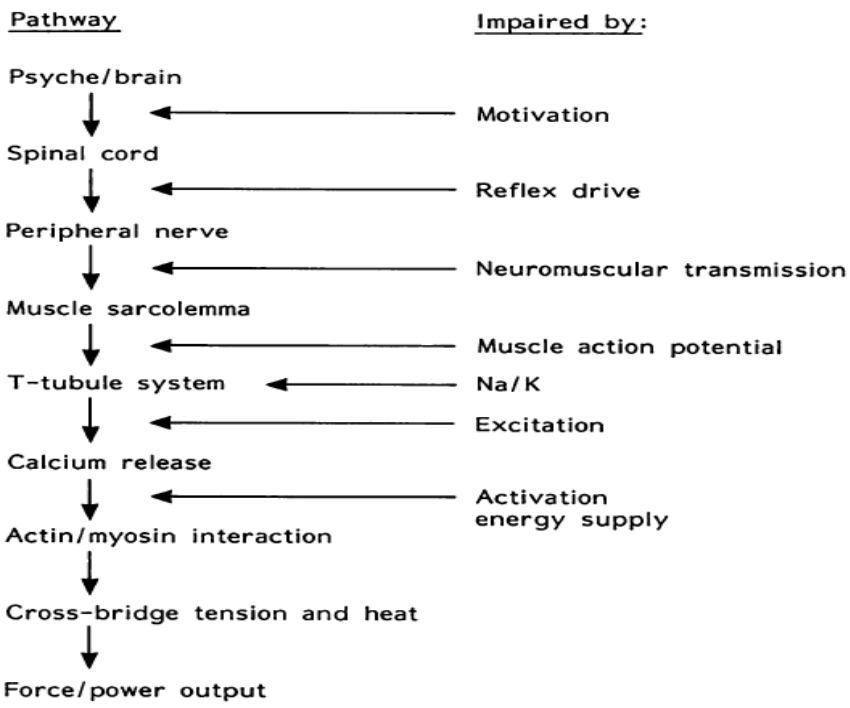


Figure 1.Command chain for muscular contraction has been drawn in Crowley et al. [5] by following Gibson and Edwards.

Isometric force produced muscle fatigue data are incomplete on the effects of temperature [1]. Several experiments have been performed showing in humans and in animal models that properties altered by decreasing temperature including voluntary isomeric maximal force. Drinkwater and Behm found that the rate of isometric twitch and tetanic force development declined by a similar 50 and 46%, respectively, when the plantar flexors were cooled to 22°C [14]. Davies et al. [28] reported that local cooling to 24°C of the triceps muscle complex reduced its maximal voluntary contraction (MVC). De Ruiter and De Haan electrically activated human adductor pollicis muscle found that concentric part of the force-velocity affected by cooling than eccentric part [29]. Oksa et al. [30] found reduced maximal muscle performances during dynamic exercise performed at a lowered room air temperature (10°–5°C) and Ranatunga et al. [31] summarized that maximum voluntary tension remained relatively stable on cooling to 25°C but varied by about 30% on cooling to 12°-15°C. By contrast, Meigal et al. [32] reported that exposure to cold (10°C) room air for 30 min did not impair MVC during isometric elbow flexion. Bergh and Ekblom also not found any difference in the EMG activity of vastus lateralis, semitendinosus and biceps femoris muscles in one cooled test the subjects with jumping exercise [33]. Sargeant in 1987 found in his study that after warming the leg in 44°C water the maximal contraction force and peak power were increased [10]. So it was expected that electro mechanics time of muscle [9] would be elongated at lower muscle temperatures due to the impaired contractile properties.

1.2. Muscle Strength Measured by Handheld Dynamometer

The hand-held dynamometer is a simple and objective tool for measuring muscle strength. Presently, this is being extensively used as this is a portable digital device incorporating a calibrated load cell [34] and relatively inexpensive comparing with others isometric strength measuring tools [35]. Spink et al. [36] considered that hand-held dynamometry is a reliable instrument with high intra-rater and inter-rater reliability to measure the foot and ankle strength of young and older adults [34] and effective for future exercise intervention studies. Some studies have shown that ankle dorsiflexion, plantar flexion and toe plantar flexion strength are strongly correlated with walking speed [36]. Hand-held dynamometer was found to be reliable for testing different muscle groups including ankle joint to record plantar flexion, inversion and eversion by the different assessors on specific target

populations. That study assessed 41 healthy older people, mean age 76.1 years with a history of falling [37] and other study assessed single test including ankle dorsi /plantar flexor diagnosed with different complications whose age range 17-82 years [38]. Bohannon confirmed that reliable measurements of muscle strength can be obtained using a hand-held dynamometer and it would be valid if the tester can produce counter by holding vertically against the patients' force [39].

1.3. Electromyography (EMG)

Electromyography (EMG) is a method to objectively evaluate muscles function [40, 41]. EMG systems detect and record the compound signal generated by the many changes and muscle electrical potentials during contraction and the process signal can be used to investigate movement co-ordination [42, 43]. EMG may be used to understand muscle mechanics, muscle physiology, neurophysiology and motor control mechanisms during human locomotion [44].

The EMG registers electrical potentials from muscles using three techniques namely, surface, fine wire and needle electrodes. The use of surface electrodes is a non-invasive method to detect superficial muscle activity. Fine wire and needle electrodes are invasive and are used to record the deep muscle electrical activity and selective motor unit activity. [43]. There is generally a positive linear relationship between EMG amplitudes and muscle voluntary force [45]. EMG studies of muscle fatigue generally assess frequency changes within the processed EMG signals [43]. Raw EMG amplitude values are generally specific to each electrode placement and thus it is difficult to use this unit to compare the EMG activity between different muscles. 'Normalization' is a procedure where EMG amplitude values are expressed as a percentage of a reference EMG recording obtained during a maximal or sub-maximal contraction, and this ratio may be used to compare muscle activity between different muscles [46].

Temperature may have a modulating effect on input and output motor control mechanisms and thus may affect EMG responses. Some authors have reported effects of cooling on EMG activity of muscles during isometric and sub-maximal exercise. Petrofsky & Lind [47] and Bigland-Ritchie et al. [48], De Luca [49] investigated the effect of cooling on EMG frequency

of the working muscles focusing on isometric and sub-maximal exercise and they reported increased amplitude of surface electromyogram (EMG) but decreased center frequency of EMG power spectra with decreasing muscle temperature and fatigue from 40° to 10°C water cooling [47]. Subnormal and fatigue muscle both have to recruit more fibers to maintain the required force level to perform a given output [50]. But the relationship between force production and EMG-activity was remained the same during cooling by the study of Holewijn & Heus in 1992 [7].

Asmussen et al. [51] focused the agonist muscle and Oksa et al. [20] found that elastic properties of the muscles through stretching were more prominent by cooling that could affect muscular performance. During shortening phase the EMG activity of the agonist decreased and the activity of the antagonist increased. After cooling during the shortening phase of the jumps the EMG-activity of triceps surae muscle complex decreased in the study. As a result, alterations in motor unit recruitment could be responsible for the prolonged muscle contraction due to recruiting more fibers and decreased force production [20]. Contractile properties of muscle such as voluntary force, electromyography (EMG) [2] were studied but little information was explored about EMG activities and ground reaction force of gait muscles in relation to temperature.

1.4. Ground Reaction Force (GRF)

Force platforms are generally used to measure force, power and velocity of human performance derived from ground reaction force time data during gait and counter movement jumping [52]. Walking performance over the force plate floor is the manifestation of human sensory motor system and acquires the gait biomechanics [65]. A single study in cold by Oksa et al. [20] measured the agonist and antagonist muscle pair of the lower leg to determine the duration of stretch and shortening phases during the stretch-shortening cycle using reaction force and EMG between the exposures to 27° and 10°C. Hori et al. [52] tested the reliability of ground reaction force plate to measure the performance during countermovement jumping that confirmed in measurement of the peak power, force and velocity of jumping. But Bassey et al. [53] observed significant relationships between ground and implant forces, where implant forces were very much higher due to muscle activity than the ground forces. Vertical with braking and propulsion ground reaction forces

and lower limb EMG activities were analyzed in a neurologic trial during gait. They revealed altered plantar sensory information by wearing shoes which did not attenuate vertical forces in non-diabetic adults with diabetic neuropathic participants [54]. One study assessed the effects of fatigue and stance width on ground reaction forces and EMG recorded trunk motion following back muscles during an asymmetric, repetitive lifting task [55]. Another research observed combined EMG and ground reaction force plate to quantify postural control and isolate the muscular activity [56]. Mäkinen et al. [57] compared 25°C general cold exposure to 10°C in 90 min long, which induced cold thermal sensation, thermal discomfort and increased muscle tone which was significantly responsible for postural sway. Piedrahita et al. [18] experimented muscle activity in 15°C of cold water for 30 min though Eils et al. [58] used icy cold water (0°C) aimed reducing cutaneous information of the plantar surface of the foot for 10 min in the right foot to analyze force plate measurements; EMG measurements and three-dimensional movement during walking before and after immersion. Also Stål et al. [59] in their studies experimented ice water to get the hypothermic anesthesia for 20 min cooling in the feet to find out mechanoreceptor activity to postural control over force platform. Currently, required coefficient of friction (RCOF) is being used in many research which represents the minimum friction needed at the foot or shoe and floor interface to get non-slip human movements. This RCOF is typically measured in dry conditions with a force plate and it has been considered as an important biomechanical parameter for determining slips and falls. [60]. No research has been reported if lower leg gait muscle cooling affects RCOF and subsequently slips and falls.

Since no studies have investigated the relationship between the ankle muscle strength, ground reaction force during gait and EMG assessment before and after cooling. Even present literature has not been documented the relationships among ankle muscle strengths, ground reaction forces during gait and EMG in relation to muscle cooling. Before commencing this intended study no trials have been done for 20 min duration of cooling with 10°C water temperature and ankle maximal muscle performance measured through dynamometer, EMG and gait ground reaction force. Some investigations have had dealt with the role of local temperature on skeletal muscle performance, yet its' effects on neuromuscular function through gait remain unexamined.

1.5. Aims

The purpose of this present study was to assess the effects of cooling on the muscle performance through maximum force, EMG during low dynamic and static contraction and ground reaction forces during gait.

1.6. Objectives

- To evaluate possible change in EMG activities and the maximum ankle dorsi and plantar flexor muscle forces during maximal isometric contraction after cooling.
- To explore the ground reaction forces and EMG changes during post cooling gait of the lower leg muscles.

1.7. Hypothesis

It was hypothesized primarily that, consequences of cooling and fatigue on muscle characteristics, the changes of maximal force in the fatigued muscle would reduce and affect EMG activities of specific muscles of the lower extremities (Tibialis Anterior, TA and Gastrocnemius Medialis, GM). The secondary hypothesis was that local cooling would modify the gait strategy in anterior-posterior, vertical components of the ground reaction forces with required coefficient of friction, and reduce electromyography (EMG) activity during walking.

2. Methods

2.1. Study Design

This empirical study was a within subject design which was exploited by isometric maximal contraction in supine position; force plate measurements; and EMG measurements which were performed simultaneously during MVC and walking before and after immersion of cold water. Independent variable (treatment) was local cooling; Repeated measurements: pre and post cooling. Dependent variables (measurements) were max forces, GRFs including RCOF and muscle electrical activity.

2.2. Participants

The study sample comprised of 16 participants, both were equal number in gender. The participants were recruited from students at Lund University by advertisement. Participants were all clinically healthy [59], no neurological or musculoskeletal abnormalities with no previous history of lower limbs, joint and muscle injuries [18, 20] which may influence normal walking and balance ability. At the time of the study, none of them were involved in any specific training program. Subjects were requested to abstain from any alcoholic beverages and intensive physical exercises 24 hours before the test. Full explanation of the purpose of the study and experimental procedures was described as well as the possible risks and discomforts involved. After taking history of medical information by general questions, all volunteer participants signed the informed consent form to enroll the experiment. Their physical and anthropometric characteristics are given below in Table 1.

Table 1. Physical and anthropometric characteristics of the participants (*n* = 16), eight men and eight women, age range 21- 34 years. Data are expressed as mean and ± SD.

Characteristics	Mean ± SD
Age (years)	27.0±2.9
Body mass (kg)	66.3±9.8
Height (cm)	169.5±7.8
Body mass Index (BMI)*	23.0±2.5

*Body Mass Index (BMI) indicates the relationship between weight and height, calculation made of body weight in (kilogram) divided by body height in meter squared.

2.3. Ethical Approval

The study combined with balance and biomechanics studies and focus interviews in the project (ID no. 100026) to prevent slips and falls was approved by the Regional Ethical Review Board in Lund (EtikPrövningsNämnden, EPN), and performed in accordance with the principles of the 1975 Declaration of Helsinki for research involving human subjects.

2.4. Experimental Setup

Two experimental stations were designed for the experiment and one was in a 'room temperature environment' (air temperature was about 21°C, air velocity was <0.5 m/s and relative humidity (RH) 30%) where the walkway with force plate was placed. In this condition, the clothing was indoor cloths like T-shirt with three quarter or trouser folded preferred by the subjects.

And other key experimental station was in the 'cold chamber' (See Figure 2), with a chair and water container (60 liters) put in front of the chair so that the participant can easily immerse both lower legs below knee while sitting on the chair. In this exposure subjects were exposed to the cold climatic chamber at 9.5°C that was 0.5°C lower than target water temperature 10°C [20, 32, 47], the aim was to maintain the lower leg cooling same at 10°C during the immersion time in the plastic water container. The purpose of the cold chamber was to maintain cold water temperature effect all most same in the time duration of 20 min [1, 29, 59] and to avoid warming up the water by the legs. A physical examination bed was also placed inside the climatic chamber to allow the individual for supine position during the dynamometer measurement. Skin temperatures were measured by using infrared thermometer (Agema infrared systems, Model –TPT64P, Germany).

Figure 2: The cold chamber set up with instruments used in the experiment.

2.5. Experiment Protocol

2.5.1 Cooling and Temperature Measurements

Before preceding the real experiments, pilot studies were performed thrice to see the effects of cold water and temperature changes during immersion period. Cold water temperature was increased more than 0.5°C after sometime over 10 min of the immersion time, although cold chamber temperature was 10°C. To maintain the water temperature constant (10°C) during the real experiment, climate chamber temperature was kept at 9.5°C, after two pilot trials also recorded water temperature raising due to warm from legs. So, consensus reached after discuss to keep the cold climate temperature at 9.5°C and immersion water temperature at the beginning was also kept at 9.5°C. Water temperature change during 20 min cooling was between 9.5° and 10.5°C

2.5.1.1. Before Cooling:

In experiment first of all, the subjects sat physically inactive in a chair in order to make them calm in physically and preparation. In the mean time, EMG electrodes were attached to the muscle belly of gastrocnemius medialis and tibialis anterior. Afterwards, skin temperature $Tsk_{\text{-Pre}}$ was measured in the room temperature environment. Followed by subject was taken into examination bed for isometric dynamometer test to perform the isometric resisted test to cold climate chamber where the bed was placed at dominant ankle dorsi and plantar

flexion with straight legs, and then walking over the ground reaction force plate with EMG. Before the tests the subjects thoroughly practiced these two movements in order to avoid any confusion or training effect during the experiments.

2.5.1.2. Muscle Cooling:

Before immersion, the subjects prepared by wearing necessary cloths for climatic chamber at 9.5°C cold temperature [20] where the water bucket was placed. After that water proof adhesive tape used over the EMG electrodes to prevent water influx into electrodes and to avoid loss of cooling effects if would needed two times electrodes placement. The cold exposure at 9.5°C aimed at local cooling of the muscle on both lower legs. In the cold chamber, the subjects were dressed light winter jackets and three quarter or trouser folded above the both knees, if needed more clothing a pair of mukluks, a pair of gloves, and a toque were provided for the individual requirements to avoid body cooling [61] and maintain core body temperature [50]. The cold water was taken approximately four times more than leg volume to keep 10°C in the water bucket to prevent warming through the body. Also to prevent water overflowing from the bucket after legs immersion, water volume was kept less than total bucket volume. Twenty minutes [1, 29, 59] were chosen for both legs placing in cold water to achieve the cooling effect. Water level was covered the whole lower legs from popliteal area to the feet in sitting on the chair. (See Figure 3.)

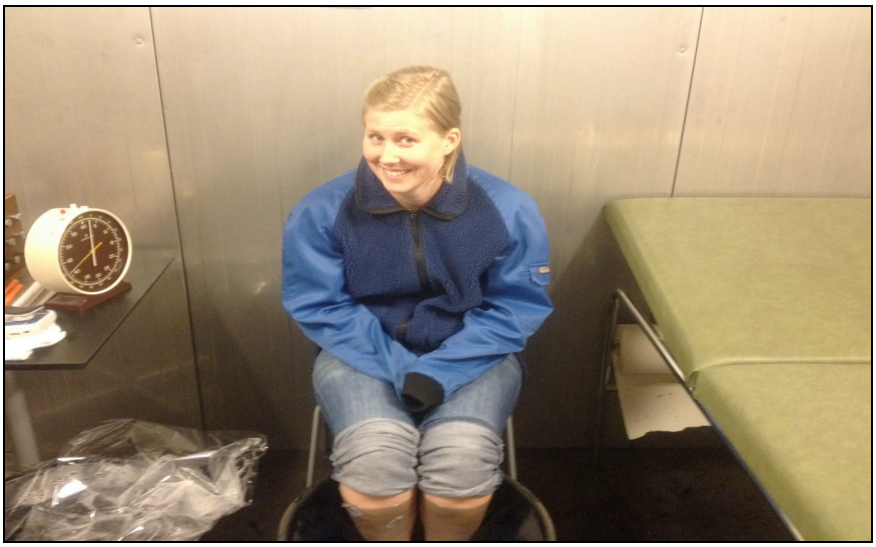


Figure 3: Subject's both legs were immersed in bucket of cold water.

2.5.1.3. After Cooling:

Again, at the end of water immersion of cold exposures skin temperature Tsk-$_{post1}$ were taken from the same spot before post cooling isometric tests in the dynamometer in the same way. Post cooling isometric dynamometer tests also performed in the climatic chamber at 9.5°C to avoid some temperature loss before walking tests. Immediately, after maximal tests Tsk-$_{post2}$ were recorded to find temperature change the subjects exposed in same 9.5°C chamber to maintain the cold effects. After measuring the skin temperature in cold chamber subjects was exposed to the ambient room temperature for post test in the first experimental station walking over the walk-way with force plate to measure ground reaction forces with EMG. Though there was loss of skin temperature during end part of gait trials over walkway in the ground reaction force plate even end cooling MVC test in dynamometer done inside of the climatic chamber. So finally, Tsk-$_{post\ 3}$ was taken at the end of post cooling gait trials at room temperature. Skin temperatures of the GM and TA measured four times in total in the same spot close to the electrodes. The test was not randomized in terms of measurements before and after cooling due to technical, time limitation of subjects' and study.

2.6. Instrumentation

2.6.1. Dynamometer

Maximum isometric voluntary force exerted by the subjects in this specific testing condition was quantified by using the 'Lafayette Hand Dynamometer' model: LA-01163 IN, US. It can measure range; on a dual scale are 0-300 pounds (136.1 kilograms) or 0-50 pounds (22.6 kilograms) and norms for ages five through adult with established protocols.

2.6.1.1. Dynamometer Procedure

All participants were assessed by one assessor to avoid any kind of differences or errors introduced by different assessors. Assessor, a physical therapist with 4 years of clinical experience, but with no experience of regular use of hand-held dynamometer practiced a number of times before the formal use. All resisted tests were performed with the participants in a supine position with hips and knees extended, and the assessor stabilized the lower limb proximal to the ankle joint to isolate the joint movement and minimize additional and substitution movements [36]. (See Figure 4).

The dynamometer was positioned against the dorsal surface of the foot just proximal to the metatarsal heads for dorsiflexion, on the plantar surface of the foot just proximal to the first metatarsal heads for plantar flexion. An extra soft cushion or towel was placed for comfort between the dynamometer contact plate and the subjects' feet to prevent from hurting. Consequently, to standardize the testing position, it was decided to test at the maximal end range of dorsiflexion by using the dynamometer to dorsiflex or extend the digits into the participant's comfortable end range of motion. For getting the maximum muscle contraction force, the ankle joint (talo-crural) was positioned in early to the mid-range in each direction of full range of motion in accordance with the optimal test position for the muscles (See Figure 4). To familiarize the participant, the assessor passively demonstrated the movement required and then asked the participants to perform the movement. Three consecutive strength of the ankle dorsiflexion and plantar flexion were assessed using the 'make' technique where the examiner hold the dynamometer stationary while the subject exerts a maximal force against it [36, 39]. Subjects were asked to build their force to maximum as hard as possible over up to 2 seconds period. By increasing force gradually in this manner it was easier for the tester to hold the dynamometer stationary against the subject's exertion. Subjects were thereafter to continue with a maximum effort for another 4-5 seconds until to get the 'beep' sound from the dynamometer. This duration showed by previous research to be adequate for the most of subjects' to reach their maximum force. One recent study with children ankle dorsi and plantar flexor, dynamometer had reported the highest reliability with the 'make' test as opposed to the 'break' test, whereby the assessor attempted to overcome the maximal effort. [36, 85]. A brief pause allowed of about 20 to 30 seconds for recovery between contractions to avoid excessive fatigue. Verbal encouragements were given during each contraction. Each movement was explained, demonstrated, and practiced with each subject in a manner appropriate to performance until the assessor felt the movement was correct and to the best of their ability. No part of the foot was touched the testing surface during the procedure and the heels rested out of the edge of the examination table [34]. Readings were taken to the nearest whole kilogram. If the difference between the scores of each was within 3 kilograms, the test considered complete, otherwise, the test was repeated.

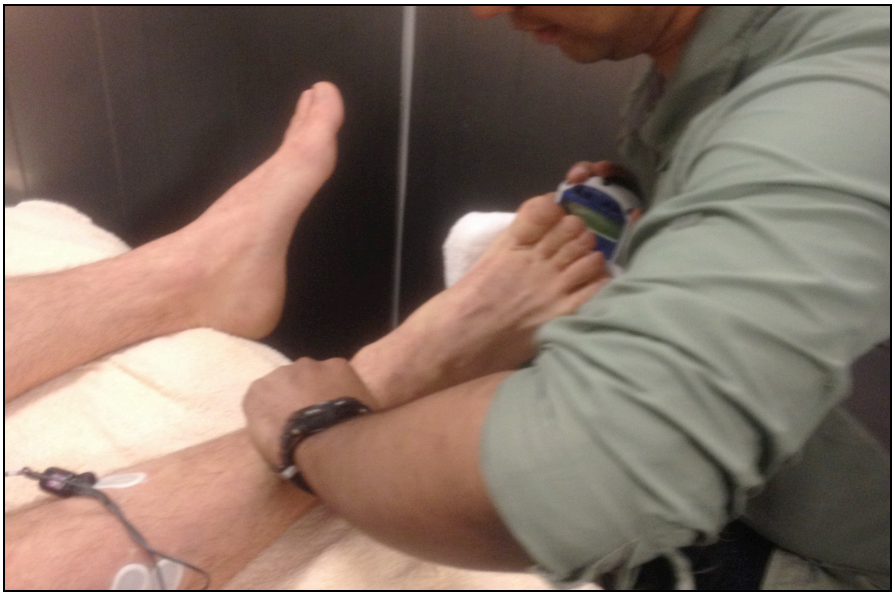


Figure 4: Hand held dynamometer was used to measure plantar flexion strength against foot resistant.

2.6.2. Walkway and Force plate

2.6.2.1. Walkway

A walkway was placed in the Thermal Environment Laboratory at the Department of Design Sciences at Lund University, Lund, Sweden. The walkway was of the following dimensions with 0.12 m height, 0.6 m wide and 7.2 m long. It was built with wooden material and covered by a vinyl sheet. The track was placed on a concrete ground-floor of the same lab (See Figure 5).

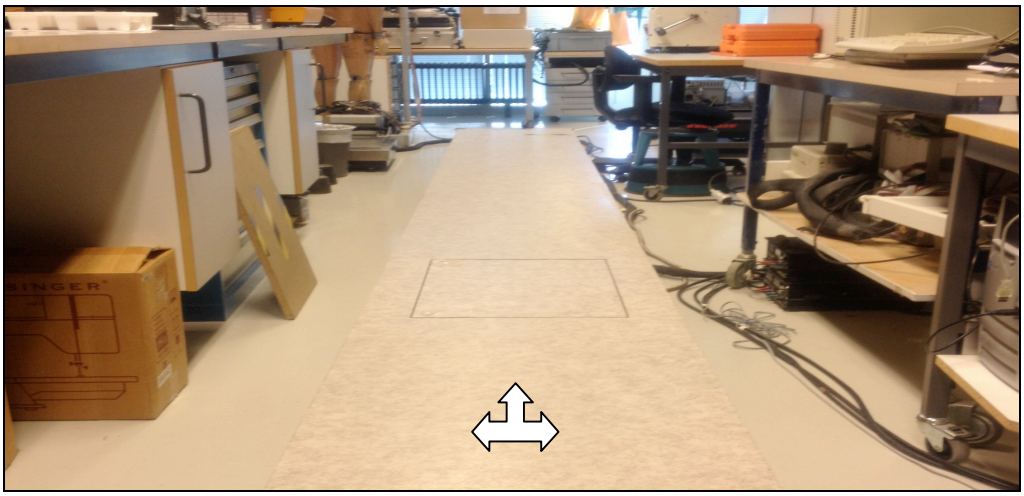


Figure 5: Force Plate was installed in the middle of the walk-way in laboratory.

2.6.2.2. Force Plate

A three-dimensional force plate 'Kistler 9281B (Switzerland) (See Figure 5) was incorporated into the walk-way. DAQ System 5695A1 with BioWare Software (Version 4.1.0.2, Type 2812A-04) used for data collection. The Force plate was the key to diagnose and analyze gait patterns. The solid metal plate rests on four pillars instrumented with piezoelectric transducers and is let into the floor so that its surface is flush with the walk-way surface. It was 0.6*0.4 m^2 and had four 3-componant force sensors built in at each corner. It usually measures the ground reaction forces for vertical (Fz), transverse shear force (Fx) and longitudinal shear force (Fy), center of pressure (COP) and moments of the forces which are calculated by 8 channels. The force plate was placed in the center of the walk-way at 4.1 m from the beginning of the walk-way track and 3.1 m from the end of the walk-way. It was also layered with vinyl sheet and flushed with the walk-way.

2.6.2.3. Gait Protocol

An overview of the experiment (general goals, walking protocol, trial types and variables recorded) was described verbally to the participant to familiarize themselves with the equipment and the testing procedure. The subject was instructed to walk at a comfortable self selected pace with normal gait throughout the experiment as naturally as possible, not to focus at the force plate or walk-way. Each subject was first given the opportunity to become familiar with the walk-way by walking across the force plate so that the dominant foot hits the force plate area by determining the starting position. Prior to each anticipation trial, the subject started walking from the starting line of the walk-way, looking straight to a picture. To be sure that the dominant foot stepped on the force plate, therefore, few trials attempted before and marked the starting point with starting foot for each subject on the walk-way. The heel strike (HS) of dominant foot during gait to toe-off (TO) from the ground in the same foot recorded automatically at the same time when a subject was stepping on the force plate.

A 'single step (SS)' walking method also used where subjects were instructed to stand close enough to the plate and walked one step over the force plate in order to obtain a high

repeatability of contacting the platform and to avoid targeting [58]. Also this might helped to evaluate the cooling effects immediately during gait before proceed to three gait trials which might took little longer time though those trials were measured swiftly direct after the cooling to prevent leg warm-up. The subjects performed totally four trials including-single step walking over the force plate both pre and post cooling.

2.6.3. EMG

Surface EMG was used to record the EMG activity of the ankle dorsi and plantar flexion both pre and post cooling. The raw EMG of the ankle dorsi and plantar flexor muscles of the lower leg was recorded. The tibialis anterior (TA) is responsible for ankle dorsal flexion (extensors of the ankle) during gait heel strike and three parts of triceps surae complex (gastrocnemius medialis, gastrocnemius lateralis and soleus muscles) all raising the heel (plantar flexion) [20] but gastrocnemius medialis (GM) was measured in this study. The 'Megawin' (ME6000-T16 Mega Electronics, Kuopio, Finland) system bio-monitor and software 'Megawin' version 3.1-b10 was used to record and analyze EMG data.

To obtain the EMG signals from above the working muscles pre-gelled bipolar surface electrodes 'Ambu Neuroline-720', Ballerup, Denmark was used. The electrodes, each with a measuring diameter of 10 mm, were taped lengthwise in the upper 1/3 over the muscle belly [62, 63] of GM ascending antero-medial part of calf and TA electrodes were placed in the ascending lateral portion 5-7 cm below from tibial plateau (See Figure 6). The inter-electrodes distance of the recording contacts was less than 2 cm [58, 62] on the muscle bellies of the GM, TA. The skin was prepared by shaving if there any hair then lightly abrading with sand paper to remove dead skin to minimize impedance below 6k Ω (ohm) [58] and finally, cleaned with isopropyl alcohol. One ground or reference electrode was placed over inactive tissue like the tibial or shin bone. To avoid changing electrodes after water immersion waterproof surface electrodes were used guarded by outlined rubber coating; further protection were ensured by attaching water proof adhesive tape over them. All electrode placements were performed by the same investigator using the 'Surface Electromyography for the Non-invasive Assessment of Muscles (SENIAM)' recommendation [64].

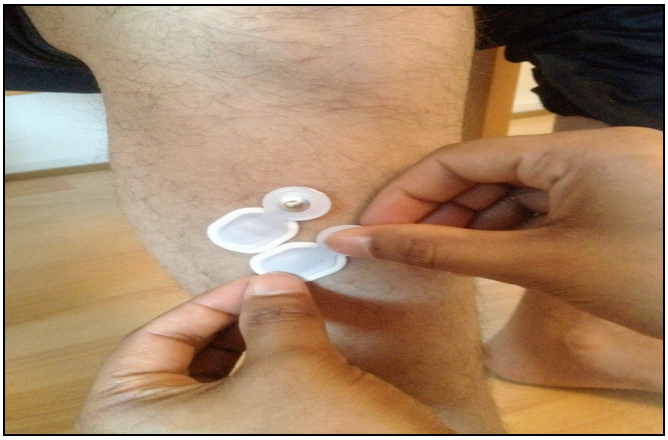


Figure 6: Surface electrodes placement over the TA muscle belly.

In order to get same movements' data from two instruments during walking over the force plate EMG signals needed to be synchronized with signals of ground reaction forces. Due to the lack of full synchronization device and time, instead of synchronizing with EMG, markers were placed during start and end of whole period of maximum contraction test. Also each gait trial from start to end by passing the force plate stance phase both pre and post cooling. Data acquisition was made by the help of 'Bioware' software where 5 sec duration was selected from the beginning of the walkway just before the heel contact to reach the force plate finished with toe off in the ipsi-lateral dominant leg through recording of whole gait cycle. It also helped to identify the EMG pattern to follow the three or more steps EMG during each gait trial.

2.7. Data Processing and Analysis

2.7.1 Data Transformation

2.7.1.1. MVC Strength

Average of three maximum voluntary of contractions and peak one from dynamometer were considered for analysis in each of two conditions. The height and weight is the co-determinant of strength assessing MVC, comparisons were performed on both raw scores and normalized scores using the formula below [36]:

$$\text{Normalized strength} = \frac{\text{Strength (kg)}}{\text{Weight (kg)} \times \text{Height (m)}} \times 100$$

2.7.1.2. EMG

The source spectra of the raw EMG recording were visually inspected to ensure general signal quality. The raw EMG signal was processed in the following way: This sampling rate was 1024 Hz; The DC offset using the inbuilt Megawin software was applied. The raw EMG signal was digitally band pass filtered (20Hz-400Hz); a notch filter 50Hz was also applied to clean the signal from surrounding electrical appliances: the signal was then full-wave rectified and finally the root mean square (RMS) average was applied. From this processed signals the peak and median values in µV were obtained. Peak values were obtained only from the MVC trials and median values for the single step and 3 gait trials. To analyze the single step over the force plate, the median EMG recordings of TA and GM were used. The single step phase measured commenced from the point of heel strike with TA activation and finished with push off activity when GM activated. Median EMG values for both muscles were obtained from the selected time period where 50th percentile of the values could be either way. Also for each gait trial the median was obtained from three full gait cycles prior to force plate impact. This gait duration was selected from EMG recording in each gait trial between markers by identifying the onset of GM activation on the first gait cycle to the onset of GM activation at force plate contact. EMG signals also were normalized to the peak EMG values for each muscle and each participant [61] for further analysis. Finally, mean EMG activity from of the two muscles before and after cooling were calculated for comparison with vertical and longitudinal anterior-posterior GRF in the dominant foot from heel strike to toe-off.

Definitions of parameters from EMG were used for data analysis Fig.7-9.

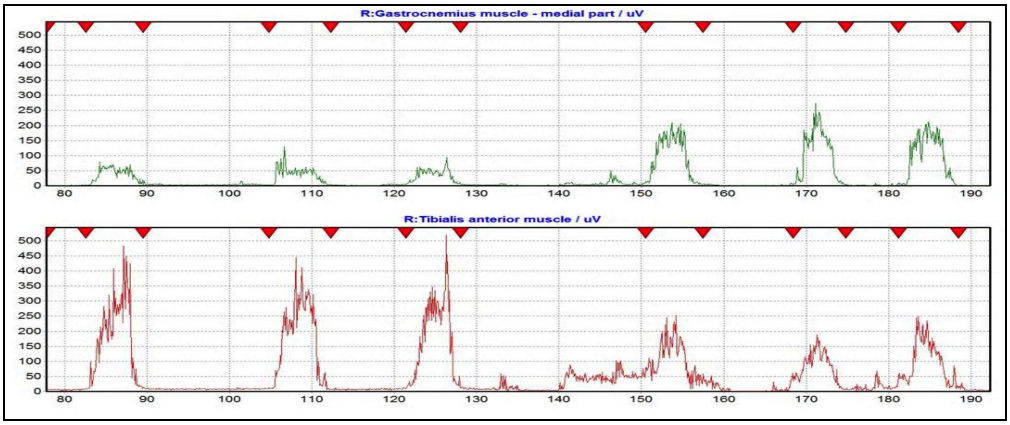


Figure 7. Example of typical MVCs from EMG. Six MVC's period marked with marker. 'X' axis shows the duration in sec. and 'Y' axis the EMG amplitude in µV.

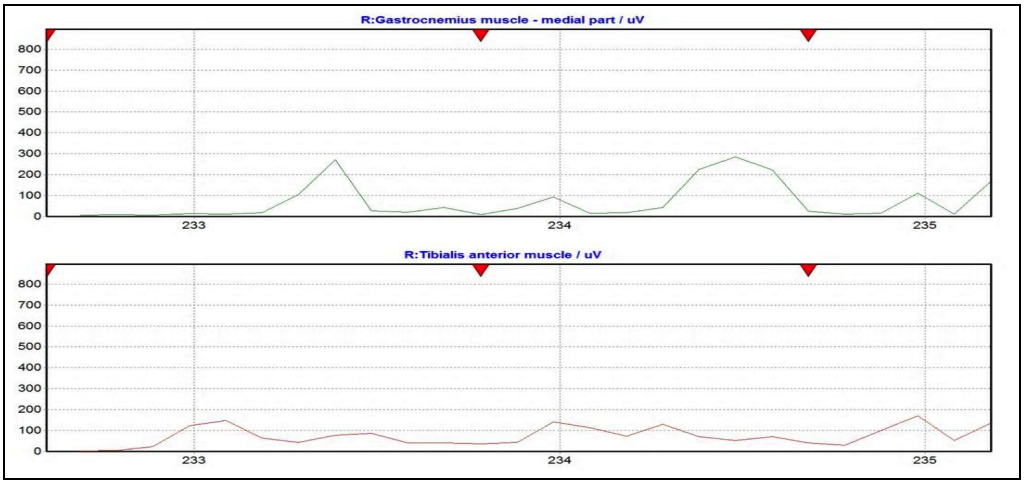


Figure 8. Example of typical 'median' EMG during single step period marked with red flags in between from EMG. 'X' axis shows the duration in sec. and 'Y' axis the EMG amplitude in µV.

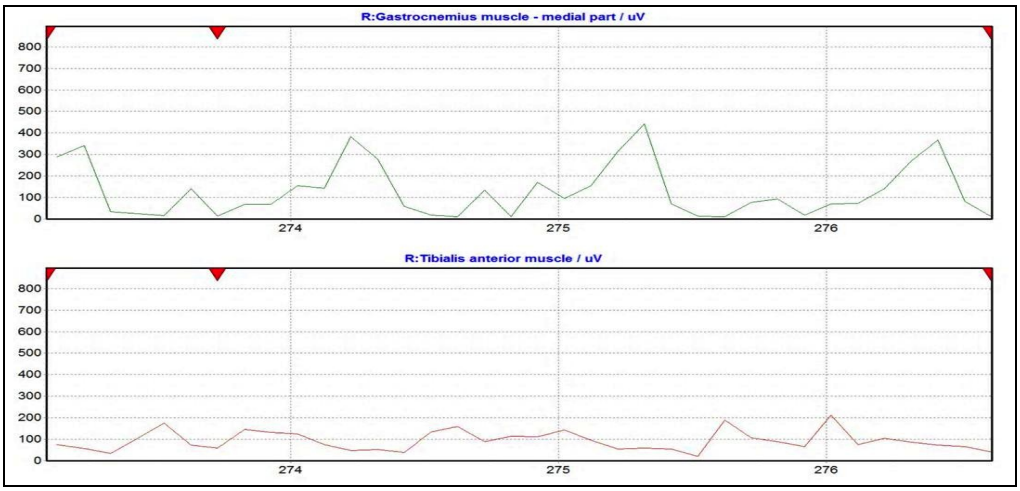


Figure 9. Example of typical 'median' EMG during 3 steps gait trial period was taken from EMG. 'X' axis shows the duration in sec. and 'Y' axis shows the EMG amplitude in µV.

2.7.1.3. Ground Reaction Forces

Three forces from the force plate, i.e. (vertical force (Fz), transverse or lateral shear force (Fx) and longitudinal anterior-posterior shear force (Fy) were obtained. The GRF data were processed using a zero lag low-pass Butterworth 4th order filter with a cut-off frequency of 100 Hz and then normalized to each subject's body weight. Time was normalized to 100% of the stance phase with 0% being heel strike (HS) contact and 100% representing toe-off (TO) from the force plate. [54, 65]. The body weight was weighed on over the force plate in order to normalize the ground reaction forces to the subjects' respective body weights.

The transverse shear force (Fx) were not analyzed because of less importance in terms of forward and backward movements of the body and ankle dorsi and plantar flexions. The sign of the longitudinal shear force indicated the direction of walking. In this study only flat walkway and straight forward walking were investigated.

The beginning and the end of the stance period was determined when the vertical force exceeded and reached 10N [66], respectively. The force plate stance phase period was divided into two sub phases (HS and TO) from the mid-point of stance phase using the time slice options in the 'Bioware' and was portrayed as a percentage. A full stance period is 100 % however, only those two sub phases were considered in the study. The HS and TO phases were associated with dorsiflexion and plantar flexion of ankle according to two force peaks in the three dimensional force curves. In HS of each gait trial, peak vertical forces (Fz) and longitudinal friction forces (Fy) were taken from the first half of period of the curve (See Figure 10).

In same way during TO, peak vertical forces (Fz) and longitudinal friction forces (Fy) were taken from the second half of period (See Figure 10). Average of all three trials HS and TO peaks were made for each subject and mean values calculated of all sixteen subjects on those two parameters for comparison in two conditions.

2.7.1.4. Required Coefficient Of Friction (RCOF)

The ratio of shear to normal ground reaction force, termed the required coefficient of friction (RCOF) [65] was calculated for each trial by dividing longitudinal anterior-posterior force by vertical force (Fy/Fz). According to Chang et al. [60] RCOF was generally accepted to the value of the third peak of the RCOF versus time curve, as shown in Figure 10 below. The third peak was scrutinized from the force plate curve by selecting the time period 20 ms to 200 ms after heel contact (10N) though sometime COFy was the 2nd peak so time period was important to find the values [60, 66].

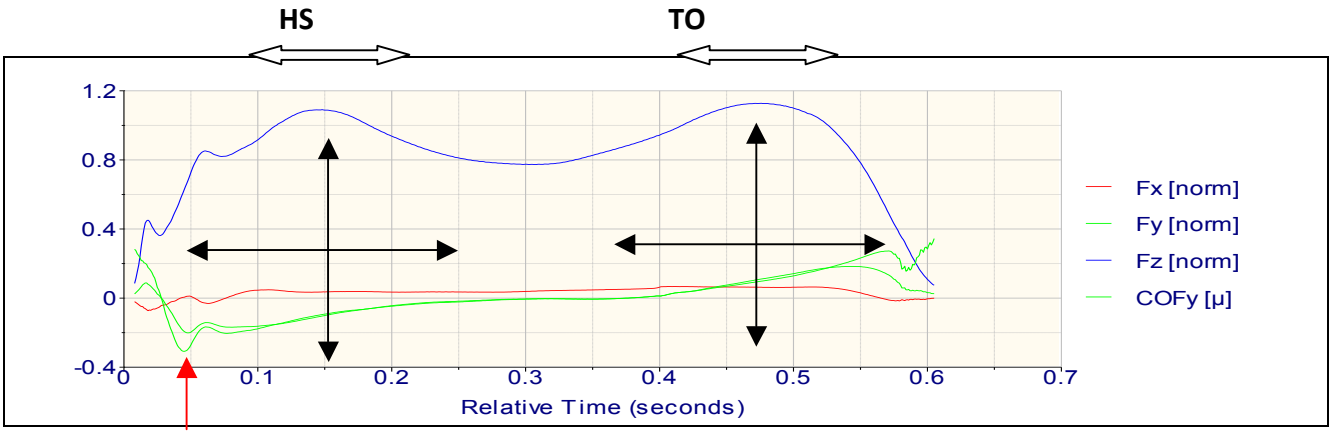


Figure 10: Example of a ground reaction forces and RCOF used for analysis, the vertical force (Fz) and longitudinal shear force (Fy) during the HS and TO phase. Peak 2 Indicated by 'red arrow' at the bottom represents the RCOF during heel strike.

2.7.2. Statistical analysis

For statistical analysis, the mean, median and max of three isometric forces, EMG and ground reaction forces and RCOF during gait trials were calculated, more specifically, both mean and max for MVCs, median EMG during three steps gait trials and single step, mean of peak ground reaction forces and RCOF. The vertical and longitudinal anterior-posterior GRF variables consisted of the two peaks during heel strike and toe-off period. The dependent variables for force plate were vertical force (Fz) during HS and TO (FzHS, FzTO), longitudinal shear force during HS and TO (FyHS, FyTO) and RCOF.

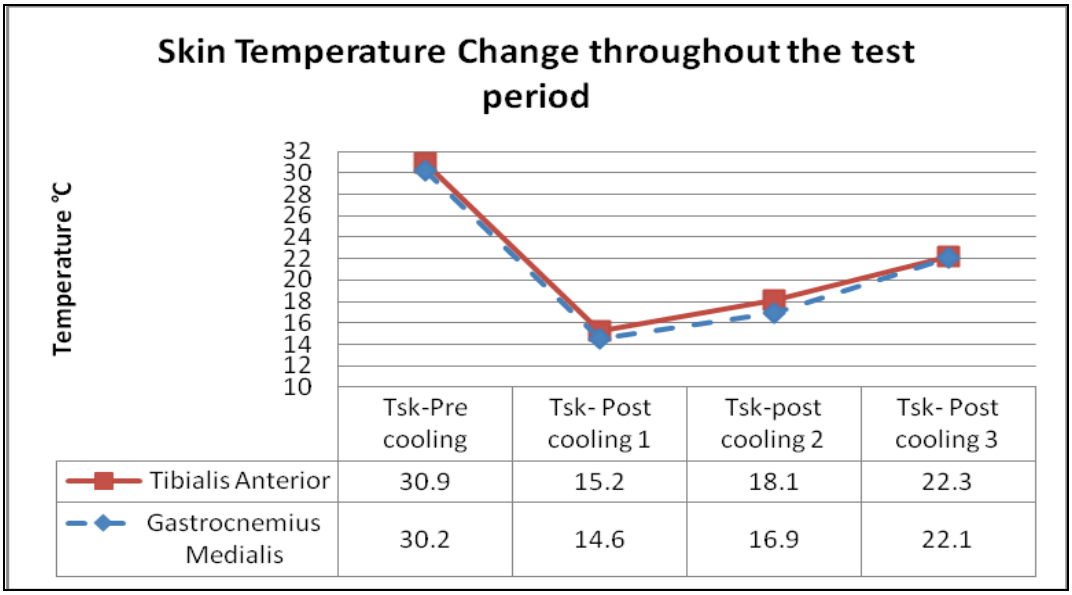
Mean ± SD values were calculated for all variables of interest. Statistical tests included within-subject 'paired t' test to assess the differences of the results measured by dynamometer, EMG and force plate between normal and cooled conditions. Since SD of EMG results are large which indicated some outliers in EMG values from few participant cases. To explore these non-normally distributed data, non-parametric 'wilcoxon signed-rank test' was performed for further scrutiny but maintain the stringency of participants' number. The statistical level of significance was set at $P<0.05$ throughout the analysis except when otherwise specified. The participants were presented by descriptive statistical data. All data were processed and analyzed using 'Statistical Package for the Social Sciences' (SPSS v.20, IBM Corp, US).

3. Results

All sixteen subjects successfully completed 20 minutes of cooling on both legs in cold water at 10℃ in the cold chamber without any drop out.

3.1. Muscle Cooling

The average skin temperatures over the two muscles (TA and GM) decreased about 15.7℃ and 15.6℃ after 20 minutes of cooling, respectively. All sixteen subjects reported reduced the sensation and bit 'heavy legs' after 20 minutes cooling. Figure 11 shows the average skin temperature changes with progression of all the experiments. Skin temperatures gradually increased about 2.9℃ and 2.2℃ for TA and GM accordingly after post cooling MVC tests, even though performed in the cold chamber. Finally, outside the cold chamber increased skin temperatures at end of all the gait trials were about 4°-5℃. The skin temperature changes over the two muscles throughout the test period are shown in the Figure 11.



	Tsk-Pre cooling	Tsk- Post cooling 1	Tsk-post cooling 2	Tsk- Post cooling 3
Tibialis Anterior	30.9	15.2	18.1	22.3
Gastrocnemius Medialis	30.2	14.6	16.9	22.1

Figure 11. Mean skin temperature over the two muscles for four measurement phases.

NB: Tsk-Pre=before cooling, Tsk-Post cooling 1=immediate after cooling, Tsk-Post cooling 2=after the post cooling MVC test, Tsk-Post cooling 3=end of gait trials.

3.2. The Effects of Cooling on Strength MVC

A comparison of the two muscles isometric forces pre and post experiments of cooling are enlisted in Table 2. Results were analyzed by taking the maximum of three muscle contractions as well as average of three attempts.

There was a significant difference between two conditions only for tibialis anterior muscle (P<.001) with dynamometer measurement in all cases of MVC's including normalized values; the gastrocnemius medialis muscle force difference was not significant. Maximal muscular force production was significantly affected through cooling mostly TA than GM. It was significant (P<.001) when mean difference between two conditions was more than 2 Kg between pre-MVC's mean and post MVC's mean; and between pre normalized MVC's mean versus (vs.) post normalized MVC's mean. Also isometric force from TA in between MVC's pre max and post max of three contractions has been reduced on an average more than 2 Kg due to cooling, and same in MVC's pre max and post max normalized. In GM, mean difference after cooling was less than 2 Kg in all cases which were not significant difference (See Table 2, MVC).

3.3. The Effects of Cooling on EMG during MVC

Peak and mean EMG for the GM was significantly higher in post cooling (P =.002 and .003) respectively and there were no exception in the non-parametric test too. The EMG amplitude had a tendency to be higher after cooling in most cases. Mean values differed more than -400 µV (-488.0 and -420.7 peak and mean MVC, respectively) in the GM muscle, whereas TA differed only -23.0 and 3.0 µV, in MVC's peak and mean, accordingly. So there were no significant differences in the EMG values for TA muscle. (See Table 2, EMG MVC).

Table 2. The means and standard deviations of the MVC's mean and max of three trials including Norm (normalized) values as well as the peak and mean EMG recording of the three maximum values from each MVC are given for the TA and GM muscles immediately before and immediately after cooling. N=16.

MVC						
	Dorsi Flexion (TA)			Planter Flexion (GM)		
	Pre Cooling	Post Cooling	Sig. 'P' value	Pre Cooling	Post Cooling	Sig. 'P' value
Parameters	Mean ± SD			Mean ± SD		
MVC Mean (Kg)	23.8±2.7	21.3±2.7	<.001	30.6±4.5	29.7±3.0	.221
MVC Max (Kg)	24.8±2.7	22.5±2.6	<.001	31.4±4.7	30.5±3.2	.287
MVC Mean (Norm)	21.5±3.7	19.2±3.1	<.001	27.9±6.0	26.8±4.7	.135
MVC Max (Norm)	22.5±3.8	20.3±3.1	<.001	28.7±6.1	27.7±4.9	.216

EMG MVC								
	Tibialis Anterior				Gastrocnemius Medialis			
	Mean ± SD		Sig. P value		Mean ± SD		Sig. P value	
			't' test	WSR test*			't' Test	WSR test*
MVC EMG Mean (µV)	446.4±218.2	443.4±250.7	.939	.210	284.8±128.3	705.5±487.7	.003	.004
MVC EMG Peak (µV)	474.9±221.6	497.9±285.2	.656	.210	321.3±147.5	809.4±534.3	.002	.001

*Non-parametric 'Wilcoxon Signed-Rank' Test for EMG data.

3.4. The Effects of Cooling on the EMG of Gait Muscles

The analysis of walking performance (See Table 3) in the EMG showed that, single step (P=.037) and three steps average median in gait trial (P<.001) parameters including its' normalized parameter were significantly affected in GM muscle after cooling except normalized single step (P=.129). Conversely, TA showed no significant changes in cooled muscle in any of the parameters. Further analysis with 'wilcoxon signed rank test showed

same results except no significant difference in either of single step median and normalized median parameters, P=.607 and P=.302, respectively. As already observed higher EMG trends of cooled muscle in MVC this continues also during GM single step's EMG. Compared with normal to the cooled GM muscle EMG activity was higher in gait trials which can be observed through results including normalized EMG. Even the same EMG pattern was continued for TA but cooling effects was not significant (P=.230, and P=.214) in single step and gait trials, accordingly.

Table 3. The means and standard deviations of the median EMG during three gait trials and their norm (normalized) values are given for the TA and GM muscles before and after cooling. Also given are the corresponding single step values. N=16.

EMG during Gait trials								
	Tibialis Anterior (TA)				Gastrocnemius Medialis (GM)			
	Pre Cooling	Post Cooling	Sig. 'P' value		Pre Cooling	Post Cooling	Sig. 'P' value	
Parameters	Mean ± SD		't' test	WSR Test*	Mean ± SD		't' Test	WSR test*
Single step EMG Median (µV)	29.4±13.1	38.5±26.2	.230	.302	16.9±9.5	35.8±29.7	.037	.607
Single step EMG Median Norm (%)	7.3±4.3	9.5±6.3	.107	.302	10.3±4.0	4.0±9.7	.129	.302
Gait trial EMG Median (µV)	46.1±17.6	52.5±21.0	.214	.077	42.3±37.6	59.1±38.4	<.001	.004
Gait trial EMG Median Norm (%)	11.3±5.3	13.1±7.3	.158	.077	13.6±10.3	19.8±11.9	<.001	.004

*Non-parametric 'Wilcoxon Signed-Rank' Test for EMG data.

3.5. The Effects of Cooling on Gait Ground Reaction Forces

The results for vertical (Fz) and longitudinal (Fy) GRF also required coefficient of friction (COFy) Fy/Fz are presented in Table 4. There were no significant effects of cooling examined in the statistical analysis of vertical GRF in walking stance phase of heel strike and toe-off in relation to TA and GM muscles. The third peak of coefficient of Friction (COFy) showed no significant difference (P=.851).

Table 4. Vertical and longitudinal Ground Reaction forces (normalized by body weight) during heel strike (Tibialis Anterior) and toe-off (Gastrocnemius Medialis) in relation to dorsi-flexion and plantar flexion respectively before and after cooling. N-16.

Ground Reaction Forces (GRF)						
	Heel Strike (HS) Phase (DF)			Toe-Off (TO) Phase (PF)		
Forces	Pre Cooling	Post Cooling	Sig. 'P' value	Pre Cooling	Post Cooling	Sig. 'P' value
	Mean ±SD			Mean ±SD		
Peak Vertical (Fz)	1.14±.11	1.17±.10	.140	1.15±.08	1.14±.06	.186
Peak Longitudinal (Fy)	-.21±.05	-.21±.06	.903	.24±.03	.23±.03	.129
Required Coefficient of Friction (RCOF)*	.26±.04	.25±.05	.851			

*RCOF during heel strike phase was included in the analysis.

4. Discussion

The present study investigated the effects of cooling on voluntary ankle maximum isometric force, ground reaction forces and EMG activity of the TA and GM during MVC trials, single stepping and gait trials. So far, to the best of my knowledge, it was attempted first combining maximum isometric force with ground reaction force and EMG to evaluate the effect of cooling on lower leg muscles. The main findings showed that the cooling decreased maximum force of the TA but not the GM muscle. The study also showed that cooling increased the EMG amplitude significantly of the GM but not the TA during the maximum voluntary contractions, single stepping and gait trials. These results partly supported initial hypothesis that maximum lower leg muscle force decreases through ankle maximal voluntary contractions.

4.1. The Cooling Effect on Ankle Muscle Strength and EMG

A significant decrease in MVC strength was observed from pre to post cooling for dorsi-flexion (DF) only, whereas, EMG amplitude for plantar flexion (PF) in GM muscle significantly increased. This might be induced by muscular fatigability as it was proved that fatigued muscular EMG during isometric contraction increased the amplitude [47]. In the cooled condition, during concentric contractions [29], the level of co-activation (i.e. increased EMG amplitude of flexor and extensor muscles), but the amplitude of EMG may be affected by many other factors: cooling may modify the shape of the action potential, and cold skin and muscle may act as a low-pass filter [50]. Rissanen et al. [16] observed that cooling increased the level co-activation of lower leg muscles at the beginning of exercise. Potential reasons for these differences may include experimental factors such as specific instructions given to subjects, different cooling duration and cooling temperatures, different body positions during max contraction. Despite these factors, the relative differences between the three MVCs and gait trials remained the same.

The results reported here were comparable to findings of earlier studies. Previous studies have demonstrated that cooling affect at peripheral muscles where isometric force production start to diminish on any of the temperature below 25°C [1, 28]. Holewijn and Heus [7] also monitored a collapse of maximal grip force after 30 min of local cooling at 15°C. Study performed to determine the effects of environmental cooling on force production in the thigh muscles observed where quadriceps and hamstrings significantly dropped performance when the environmental temperatures at or below 10°C for at least 40 minutes. In the mean time, body losses a significant amount of heat as environmental temperature decreased indicating performance reduction and warm-up time will increase too [67]. We have only shown the TA significantly decreased its' performance. Possible reason for the discrepancy of GM MVC was not affected might be that, GM is much larger muscle than the TA which may imply that the drop in core muscle temperature of this large muscle was less affected by the cooling method of the study. However, the surface skin temperature did decrease significantly but more invasive techniques are required to register the core muscle temperature. Another reason why MVC was not affected that PF also performed by the large and deep Soleus muscle. The soleus muscle is known to be rich in blood volume which may resist superficial cooling effect. Cè et al. [22] purported after

muscle cooling, decreased conduction velocity in low temperature, especially, combined and additional effect of cooling and fatigue changes the sarcolemmal propagation properties thus altering force production. Oksa et al. [68] disputed that muscle activation changes due to cooling and rewarming are expected to centrally regulate due to muscle agonist/antagonist pattern changes followed by cooling. Agonist and antagonist muscles needs to play a momentous role during production of maximal force through control of the function during gait [20]. All the reports concentrating from various studies on EMG amplitude of the fatigue and cooled working muscles observed increased amplitude on isometric and sub-maximal exercise due to recruiting more fibers to produce same force after fatigue but decreased center frequency of EMG power spectra with decreasing muscle temperature and fatigue from 40° to 10°C water cooling [47-50].

Individual muscular characteristics and morphologic structure could be the factor for resulting performance in PF, gastrocnemius medialis. Muscle TA mostly composed of type-1, 70% slow oxidative, non fatigable fiber where as GM, however 50% are type-2 which are fast oxidative glycolytic (FOG) or fast glycolytic (FG). Type-2 fibers due to their metabolic properties are more fatigable and it is generally accepted that EMG amplitudes increase during fatigue. It is possible that the cooling as performed in the study exerts stress on fast fibers thus resulting in higher EMG amplitude in GM muscle but not the non fatigable fibers of the TA muscle. [69]. The decrease in DF isometric strength was at least in part attributed to failure of excitation–contraction coupling and the effects on sarcolemmal propagation properties as suggested by the low-frequency fatigue [22]. These results contradicted hypothesis that both DF and PF would be fatigued after cooling. Previous studies have revealed that PF was more prone to fatigue after a prolonged level running [63]. Another observation made by Swanson and Caldwell that the EMG amplitude of the gastrocnemius and other PF muscles during the support phases was greater [70].

There were different patterns of the results of MVC between the dynamometer and EMG. Dynamometer showed that force reduced after cooling but the EMG showed the opposite trends. So there was pattern of results expression on the effects of cooling measured by these two instruments and possible different muscular characteristics and functions of those two studied muscles. Also GM was only studied muscle in EMG in this study but not the only

muscle responsible for PF measured by dynamometer for MVC tests. Moreover, TA has got an optimal muscle fiber length over two times the length of GM which allowed itself more favorable force-generating contractile affordability compared with the plantar flexors as walking speed increased studied by Neptune and Sasaki [71]. Hreljac reported that the dorsi-flexor muscles perform at or near their maximum capacity when transition of speed required and may be susceptible to overexertion [72]. TA is more favorable than the plantar flexors to generate force; however, TA is activated far below its maximum about 60% compared to 100% activation of the plantar flexors. This is applicable for normal conditions but after cooling already exerted muscle would be more prominent. The differences in levels of muscle activation and over-exertion of the plantar flexors might involve to the mechanical energetic influence on walking [71] which supported the result of this study regarding EMG and GRF. Neptune et al. [73] argued TA is primarily responsible for helping to toe-off in early swing, while the plantar flexors support body to forward progress and swing initiation in late stance. Recently, published study by Fourchet et al. [63] showed DF is less subjected to fatigue during a prolonged 5 hour hilly running than that of PF.

4.2. Muscle Cooling and Ground Reaction Forces during Gait

The analysis of ground reaction force showed no significant alterations on peak vertical and longitudinal ground reaction forces. But the little decrease can be seen from vertical GRFs during the heel strike and toe-off phases also decrease in the longitudinal anterior-posterior GRFs during the heel strike phase. This suggests that the ability of the muscle to produce force reduced as walking speed approaches to the initiation of next gait [71]. The findings related to the moments at the lower extremity joints suggested that the knee and hip appear to be used more than the ankle to control. Sacco et al. [54] in their study showed that walking with shoes changed the motor strategy for both control and diabetic participants with a higher vertical peak GRF at initial ground contact with a higher propulsive force. Eils et al. [58] and Stål et al. [59] aimed to study another strategy with developed the anesthetic effect on feet by using ice water and found the gait changed on force plate, where timing of first (HS) and second peak (TO) was modified by delaying and braking and acceleration forces were reduced. As this study also used cold water there might have reduced the cutaneous sensation that could affect the result of RCOF with cautious gait and stepping [65]. But Stål et al. [59] reported that anterior-posterior and lateral torque

response to the vibratory stimulation was higher with cooled feet though almost equal after about 2 min. The results of this study concerning the RCOF and floor effects with the expectation that subjects were aware of relatively small risk to slip over dry vinyl floor surface. These occurred due to instructions given to the subjects to walk at normal speed as much as possible during both conditions. RCOF is the ratio of Fy over Fz and is used to assess slip risk. If available coefficient of friction in GRF is smaller than RCOF, a slip will occur. Reductions in the relative magnitude of the shear forces was more than the vertical forces resulted in the overall effect of a decrease in the peak RCOF [65].

The results of this study concerning the cooling effects were consistent with the hypothesis in matter of muscular performance except sophisticated ground reaction forces. These non significant changes of ground reactions forces after cooling might be explained by the tasks and forces that were not demanding during walking. Gait speed could be an another determination, subjects were asked to walk only a few steps so not much high speed required to complete a trial. We do not know if there is any fast gait speed or running that could make a high impact on the force plate. It seemed that the non-max performances by the muscles were not much affected by the cooling which requires further research. There are some reasons could be possible explanation of supporting these result. The method of cooling which might cold the whole lower leg nerve**,** also neuromuscular junction (end plates), calcium ion flow, contractile protein etc which might slowed nerve conduction velocity [2,9,6,47]. A few instigators also pointed out increased joint stiffness [10] due to increased viscosity of the joints' synovial fluid by LeBlanc [74] after cold water exposure. This may also alter postural control and force production through joint too, consequently hampered EMG amplitude. Sekihara et al. [75] pointed out cooling tend to increase in muscle stiffness that promote resistance to high velocity movements. Though, some authors of different studies disagreed about the muscle cooling has an effect on force production [76-79] and performance [80, 81]. But the difference observation by Hopkins and Stencil [82] appears to be the result that the type of measurement used, the location of ice immersion (muscle or joint), and the time when (during or following ice application) the motor output measurement was taken. Also Miniello et al. [83] concluded no impairment of ankle stabilization following landing from a jump after cold water immersion of the entire lower leg. However, Kinzey et al. [84] showed decline in vertical impulse during a single leg

vertical jump following cold water immersion of the ankle. These authors ended up with that a decrease in nerve conduction velocity was a primary contributor to the decrease in vertical impulse. Since the average peak vertical ground reaction force was not significantly changed, the time component was primarily responsible for non-significant decrease in vertical impulse. In other words, either the extra time necessary to produce force following cooling or the muscle contraction time is slower [9, 84].

Subjective feedback was received from some of the participants about lightly reduced plantar sensation with a bit 'heavy limb' after 20 min of cold water immersion at 10°C but gradually regained during walking trials**.** In this investigation although reduced sensitivity after cooling was not explicitly focused, it is well documented that this temperature might also alter plantar sensation by stimulating the mechanoreceptors of the skin [58, 59]. After submerging the both legs in cold water, some subjects initially reported a feeling of discomfort that disappeared after a very short moment and eliminated drop out from the participation of the study, otherwise no more complain received. There were no significant alterations in sensory-motor control as measured by the force plate and EMG in the bare foot and on the dry vinyl layered surface. As they have had some reduced plantar sensitivity, it might be possible that the muscle recruitment strategy responded from afferent sensory put more extra joint loads during walking. Due to the decreased plantar sensitivity, changes in EMG amplitude following external loads would have expected.

4.3. Limitations

There were some limitations to the present study. Firstly, the skin temperatures over the muscles were measured. The muscle temperature was not directly measured. Secondly, during the resistance of the ankle plantar flexion few forces were unable to be controlled properly by the assessor. Inadequate force and stabilization by the tester may have affected the results from dynamometer. May be hand-held dynamometer was not most advantageous instruments for measuring DF and PF. A better method would be using isokinetic measurement like 'Biodex' by using band to stabilize the lower leg and knee sit on the machine. However, for practical purposes because the measurement has performed in cold chamber so hand held was preferred method. The hand held dynamometer has been

demonstrated reasonably reliable and valid in previous study [35, 36, 38]. There might have some training effect that could be a confounding factor.

Moreover, the skin temperature gradually increased in some participants' case which might have affected the present results. However, the muscle temperature would have increased more slowly considering the effect of ambient air outside cold chamber. Previous studies had similar limitations where skin temperature was not measured continuously during tests after cooling [58, 59]. Even there were some chances that cannot be ignored to affect the ground reaction forces (GRFs). The increase of the skin temperature and possibly the muscle temperature might have affected the GRFs. Because the walkway and force plate were placed in the room temperature environment and were not possible to install in the cold climate chamber in this study due to the size of long walkway.

Furthermore, single step (SS) method was used in this study to avoid quick warming up the leg muscles and check the EMG pattern as well as a try to synchronize force plate date with EMG. It has to be considered that single step will be different from steps during normal gait period due to differences in braking and accelerating the centre of mass of the body [58]. This has been observed from gait pattern between the single gait and general gait trial. However, it should be kept in mind that the present results demand real cooling effects produced from ankle during normal gait not any intentional gait. For analyzing and associating GRFs with EMG during different gait phases, synchronization was a prerequisite. Due to unavailability of synchronization apparatus EMG signals were not time normalized to 100% of the stance phase with GRFs on the force plate. Therefore, EMG markers were used during gait trials on the walkway and median EMG amplitude of three steps between the markers was used in the EMG analysis. Mean power frequency (MPF) values of the tibialis anterior (TA), gastrocnemius Medialis (GM) EMG activity were not analyzed. There could be more results if we analyze the RMS max and mean power frequency (MPF) parameters for MVC and gait trials in EMG.

4.4. Recommendations

The results of this study have some possible applied implications in future studies on muscular performance in the cold using human subjects. In cold water immersion at 10°C over 20 min TA cannot exerts its' maximum force and starts to affect its' performance. And cold water temperature close to 0°C like ice water application results could be different in matter of performance measurements even body cooling. Sport and work performance may decline due to local cooling in cold environment; subject may not produce their maximum.

The results may indicate that muscular 'homeostasis' physiological regulatory control process can be disturbed in this degree of cooling whether it is artificial cooling or outdoor exposure in winter. Ground reaction forces may be affected by the cooling during high velocity human movement. It would be interesting if it can be installed with some sports surface where athletes are using to produce force for momentum for example jumping surfaces to evaluate the performance. As it has been evidenced that cooling may be a determinant for muscle force production, recovery in warm water after cooling could have some interesting outcome. Warm water might help to recover quickly from such cold related muscular obstruction in winter activities and sports.

It is still controversial about cooling effects to induce muscle fatigue; further studies are needed focusing on fatigue muscle characteristics of repetitive movements with those activities after cooling. In the clinical application of cooling in terms of injury prevention, it appears from these results that during rehabilitation or treatment of muscular injuries there might have chance that would affect performance or function of muscle immediately after cooling. This study method can be applied to rehabilitation progressive gait analysis with some other sportsmen and patients. Ankle joint motion, angles and gait phases that could be investigated in future studies after cooling.

5. Conclusions

In conclusion, the present study evaluated plantar flexion (PF) and dorsi-flexion (DF) neuromuscular functions after 20 min of 10°C cold water immersion and found that strength loss occurred only in DF, but may fatigued, over-exertion occurred in PF as evidenced in EMG. DF-TA strength lost in post cooling force production; conversely, PF-GM in EMG expressed its effects of cooling with high electrical amplitude by getting fatigued or exhausted. Though previous studies have had some controversial findings about isometric force production in cooled muscle, this study more or less established 'impeded strength'. However, the altered maximal muscular strength did not influence so much ground reaction forces during normal walking on the dry and level surface. In other words, maximum muscle strength is impacted by the cooling of 10°C water immersion in 20 minutes, but the strength required for general or unchallenged activities is not affected, which implies that maximal performance of sports or daily vigorous activities can be affected, but sub-maximal activities can be maintained, effortlessly.

6. References

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7. Appendix

7.1 Consent and Data Collection Form

Effect of cold in ankle muscular performance in relation to the prevention of slips and falls

Background and objectives

We would like to invite you to participate in study about ankle muscle performance during walking on walkway before and after the lower legs are cooled.

Experimental conditions, measurements and procedure

The experiment will be performed in the following steps. After filling in personal information you will be informed of the test procedure. Before start surface electrodes will be applied on important muscles to record lower limb muscle activities and electromyography (EMG). Then your maximal ankle forces against a dynamometer will be tested in the laboratory. After that you will be walking on a walkway. Then the lower limb muscles will be cooled using cold water at 10 °C for 20 min. Again after cooling, you will be tested with your maximal ankle forces against a dynamometer and following walk on the walkway with the same shoes in the laboratory. After this test, we invite you to participate in a focus group interview with six to eight persons in a group on another day where you can describe your experiences that are important for designing shoes with improved slip resistance.

You will have to abstain from alcohol 24 hours and not do intensive physical exercises before the test. You should not have any neurological, orthopedic, musculoskeletal and other disorders which may influence your normal walking and balance ability.

You will be asked to attend two times on two different days, the first day for the testing in the laboratory and second day for the focus group interview, in total about one hour for each day. Data will be analyzed and presented at group level, and will not be connected to your personal information. There will be a reimbursement to cover costs. You are free to terminate your participation at any time without giving any reason.

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Consent form

I agree to participate in the above-described study.
Name: _____ Signature: _____ Date: _____
in Lund.

Email:_____ Tel:_____

Data collection sheet

Name : *Sex: M / F*

Age (years) : *Stature (cm) :*

Body mass (kg): *BMI :*

Dominant Foot:

EMG With Dynamometer

Pre test

Cont^{n} :1		Cont^{n} :2		Cont^{n} :3		Ave Cont	
DF	PF	DF	PF	DF	PF	DF	PF

Post test

Cont^{n} :1		Cont^{n} :2		Cont^{n} :3		Ave Cont	
DF	PF	DF	PF	DF	PF	DF	PF

Walking trials, Ground Reaction Forces and EMG

Pre test

Trial 1	Trial 2	Trial 3	Trial Completed

Post test

Trial 1	Trial 2	Trial 3	Trial Completed

Skin Temp

Pre Sk Tm		Post 1 Sk Tm		Post 2 Sk Tm		Post 3 Sk Tm		Ave SkTm	
TA	GM	TA	GM	TA	GM	TA	GM	TA	GM

Steps of Experiments

Before cooling

Step 1

Subjective information,
Gathering information by a simple questionnaire or questions about their health, e.g. neuromuscular disorders, cold allergies etc.
Sign consent form.

Step 2

Prepare subject for electrodes attachment like
Put off the shoes and socks, fold the trouser or pant up to knee.
Shaving leg hair, lightly abrade skin and cleaning with isopropyl alcohol.
Mark over the skin with water proof ink for EMG electrodes.
EMG electrodes attachment on two muscles: Gastroc-nemius medialis & Tibialis Anterior.
Put the water proof adhesive tape over the electrodes.
Record skin temperature.

Pre test

Information with instructions about dynamometer and demonstration of walking over walkway before connect to the apparatus

Dynamometer test

- Execution of the test and information with instructions before connect to the apparatus.
- After getting the skin temperature, during resisted test against the dynamometer developing force up to 1 to 2 sec your maximal level and hold for 3- 5 sec.
- Repeat twice more.

Walking on walkway

- Demonstration and trial of walking over the surface.
- Make sure the dominant foot will be stepping on the force plate, mark starting point on the walkway for each subject.
- Subject should walk as naturally as possible with their own pace and look forward, not to focus at the force plate or walk way.

- Perform 3 successful walking trials, and simultaneously make 3 synchronized ground reaction force and EMG recordings.

Preparation for cooling

Step 3
Remove the EMG cables from the electrodes.
Put on necessary clothes, sit on a chair in climatic chamber with air temperature at 10 °C, and immerse both lower legs just below the knees in cool water at 10 °C for 20 min. (measure and maintain water temperature at 10 °C?)

After cooling

Wipe water over the skin with towel
Remove the tape over the electrodes
Measure skin temperature over the muscles or marked skin? (to make sure the muscles are cooled or to keep the skin temperatures at a relatively same level…)

Step 4

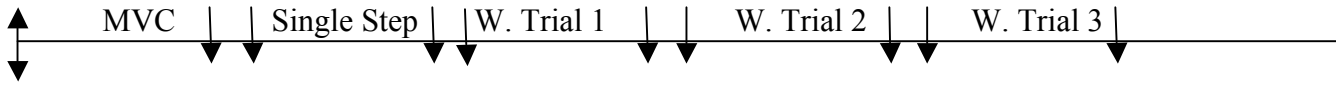
Post test
As before

Repeat Dynamometer test as before cooling.

Repeat Walking on walkway test as before cooling

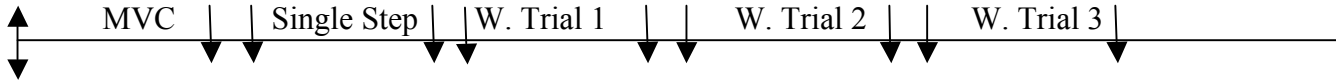
General Marker placement for EMG:

Pre cooling



Ms 1 Mf 2- Ms3 Mf4-Ms5 Mf6- Ms7 Mf8 -Ms9 Mf10

Post cooling



Ms 1 Mf 2- Ms3 Mf4-Ms5 Mf6- Ms7 Mf8 -Ms9 Mf10