

Effects of water immersion on soleus neuromuscular parameters

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ABSTRACT

Aquatic rehabilitation is a widely used tool for injury prevention or rehabilitation. The underlying neuromuscular mechanisms during immersion are less known due to methodological issues. The following study main purpose to assess neuromuscular functions and muscle architecture changes within immersed condition. The physical properties of water to the human body are well understood, as well as its effect on the cardiorespiratory system. While the Hoffmann reflex, as a tool to assess neuromuscular changes on dry land is widely examined, it has been rarely used during immersion. The effect of water immersion on muscle behavior is also unknown. In this study control values were recorded in sitting position for one hour on dry land. This recording period was needed to mimic the similar protocol as in water and to compare the two conditions. The dry land recording showed no changes in the recorded parameters over time. Unaltered reflex and muscle architecture parameters on dry land indicate that the changes during immersion are due to the altered environment. During the first 15 minutes of immersion maximal M-wave and muscle thickness decreased significantly. Maximal H/M ratio showed no significant changes in water immersed condition. The decreased potentiation of the muscle due to the architecture changes is reflected by the decreased maximal M-wave; however the unaltered H-reflex indicates background compensatory mechanisms. Possibly the activation of peripheral mechanoreceptors and the effect of weightlessness decreased the tonic presynaptic inhibition which leads to compensated H-reflex response. Repeating the protocol showed good reliability of the maximal H/M ratio, and good inter-session reliability of the muscle thickness. The thesis indicates that with adequate precaution, H-reflex and ultrasound recording is possible during prolonged immersed condition, while the results raise the possibility of acute peripheral and central neuromuscular activation during immersion. With the current protocol the precise mechanisms cannot be addressed, it rather raises the importance for future studies to measure muscle architecture and presynaptic inhibition.

Key words: aquatic therapy, immersion, electromyography, Hoffmann-reflex, ultrasonography

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1 INTRODUCTION

Aquatic therapy is a widely used, essential tool in fitness training and physiotherapy (Thein & Brody 1998). It is considered to be an effective means of active physical therapy in the rehabilitation of numerous patient groups, as well for healthy athletes and those returning from injury (Becker 2009). Water provides an excellent environment to reduce gravitational forces and loads on the joints, can be used as a resistive force for multidimensional movements, provides balancing stimulation and can be used as protective environment to reduce the risk of falling (Bates & Hanson 1996). The effect of water immersion is well studied on physiological aspects, however, its effect on neuromuscular function is largely unknown (Severin et al. 2016). The possible reason for the lack of study conducted during immersion is the methodological issues that have to take into account when using electrical devices in water. Because of this, very little work has been done to examine the underlying neuromuscular mechanisms and muscle architectural changes during immersion (Pöyhönen & Avela 2002; Sato et al. 2012; Cronin et al. 2016).

2 LITERATURE REVIEW

2.1 Aquatic Environment

2.1.1 History of aquatic therapy

Water is an excellent exercise medium due to the altered gravitational conditions and physical properties. The aquatic environment can be effectively used as a rehabilitation tool or for conditioning programs (Thein & Brody 1998).

First historical evidences dates back to 2400 BC as water was used for religious and healing ceremonies in river valley civilizations of Mesopotamia, Egypt, India and China. Greek civilizations around 500 BC recognized the benefits of water as a curative treatment developed for baths and springs. Hippocrates (460-375 BC) treated many different diseases, muscle spasms with hot and cold water immersion (Brody & Geigle 2009; Kelly et al. 2000).

Modern aquatic physical therapy dates back to 1920' when the first Hubbards tank has been developed. In 1937 Dr. Charles Leroy Lowman published 'Technique of Underwater Gymnastics: A study in Practical Application' with detailed aquatic therapy methods (Lowman 1937).

2.1.2 Physical properties of water

When a body is immersed to the water immediate and delayed biological effects are related to the fundamental principles of hydrodynamics. These properties are such as density, reduced gravity, hydrostatic pressure, buoyancy, viscosity and thermodynamics (Becker 2009; Thein & Brody 1998; Brody & Geigle 2009).

Density. The relative density of an object is the ratio of the weight of the object to the weight of an equal volume of water (Brody & Geigle 2009). If this ratio is smaller than 1, the object

will tend to float. On the other hand, if the relative density is bigger than 1, the object will tend to sink. Average density of the human body is 0.974, with women averaging lower density (Becker 2009). The relative density of a body depends on its composition - fat mass has a density of 0.9, while lean mass has a typical 1.1 density. Obese people with more fat mass and less muscle tissue have lower relative density (Becker 2009).

Hydrostatic pressure. Pascal's law states that "At any given depth, the pressure from a liquid is exerted equally on all surfaces of the immersed water" (Brody & Geigle 2009). The hydrostatic pressure is the product of the depth and fluid density, and doesn't correlate with the total weight of the water in the vessel (Wilcock et al. 2006). When a body is submerged underwater compressive force is exerted in all directions at any given depth (Brody & Geigle 2009).

The pressure at sea level (atmospheric pressure) is 1 atm/760 mmHg/14.7 psi and changes linearly with increasing depth (Figure 1). Pressure changes approximately 0.74-0.76 mmHg/1 cm, which causes different compressive force on the submerged body parts (Bove 2002; Ohanian & Markert 2007).

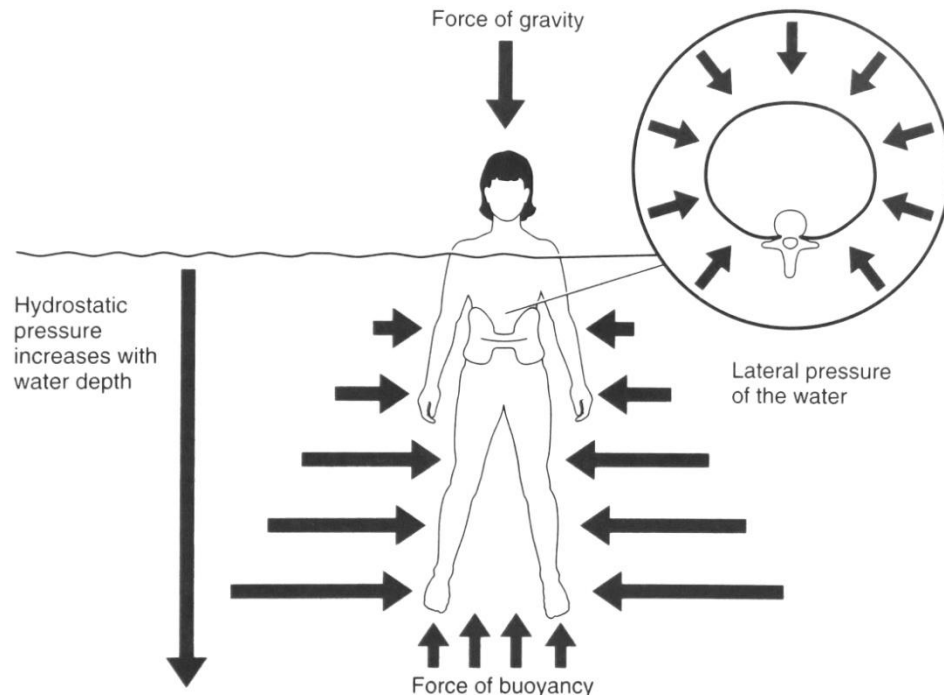


FIGURE 1. Pressures exerted on the body and the direction of gravitational and buoyancy force on the immersed body (Bates & Hanson, 1996).

Buoyancy

“The buoyant force on an immersed body has the same magnitude as the weight of the fluid displaced by the body.” - Archimedes

As Archimedes’ principle states when a body is immersed to water it experiences an opposing force towards the water surface. Buoyancy is an upward force that fluids or water exerts on a partially or totally immersed body in the opposite direction of gravity (Ohanian & Markert 2007). The force is the result of the pressure difference between the bottom and the top of the body. Buoyant force is equal to the product of the density of water and the immersed volume of an object, or the volume of the water displaced (Pöyhönen 2002).

Buoyant force acts through the center of buoyancy which is the center of gravity of the displaced fluid (Bates & Hanson 1996). In water two opposing forces act on the body: gravity, acting through the center of the gravity and moves away from the surface and buoyancy that acts through the center of buoyancy and moves towards the surface. If the two

opposing force are not in the same vertical line it causes a roll over or turn, therefore it generates a torque (Pöyhönen 2002; Bates & Hanson 1996).

During aquatic therapy buoyant forces can be used in three different conditions: assistive, resistive and supportive (Bates & Hanson 1996; Thein & Brody 1998). An assisted exercise occurs when movements are toward the surface and a resistive exercise occurs when movements are executed away from the surface. Exercises parallel to the pool and perpendicular to the upward force are supported movements (Thein & Brody 1998). These elements can be enhanced by flotation devices (Brody & Geigle 2009, Figure 2).

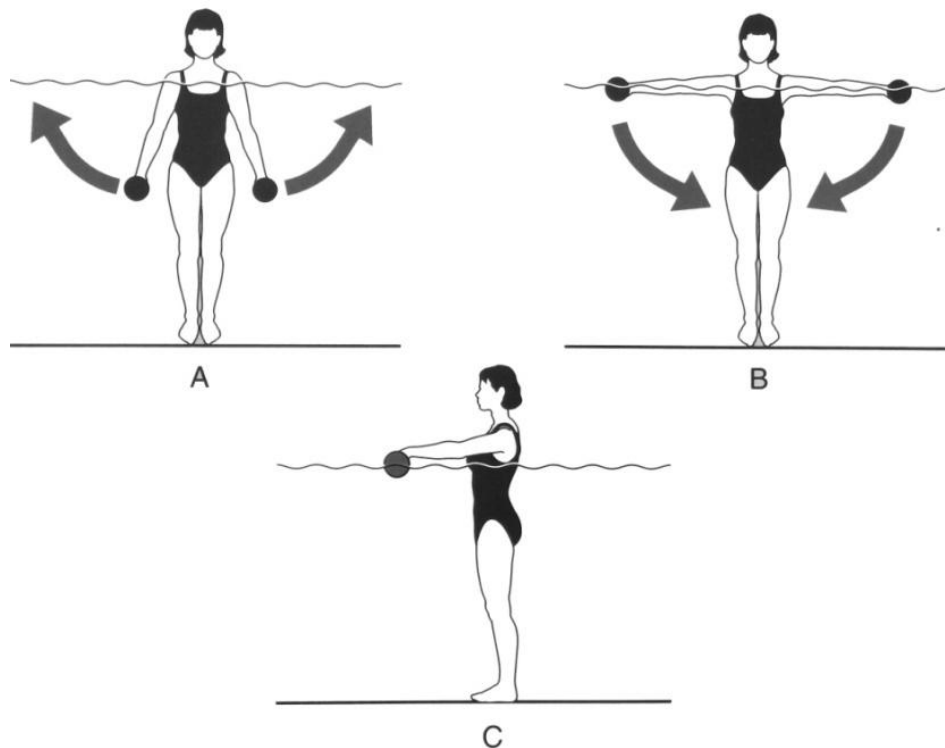


FIGURE 2. Hand-held floatation can be functionate as an assistive (A), resistive (B) or as a supportive force(Bates & Hanson, 1996).

Effect of Buoyancy in weight bearing. Buoyancy has a direct influence on the immersed body/object reducing the weight-bearing forces (Thein & Brody 1998; Bates & Hanson 1996). For static erect standing position, Harrison and Bulstrode (1987) calculated the weight bearing percentage of the human body at different immersion levels: seventh cervical

vertebra (C7), xiphisternum (XIPH) and anterior superior iliac spine (ASIS). The percentage of depth immersion against the participants' height, for the anatomical levels was: 85% at C7; 71% at XIPH and 57% at ASIS of partial immersion (Fabricius 2011).

Male athletes' percentage weight bearing was 8% at C7, 35% at XIPH and 54 % at ASIS level. Female athletes' percentage weight bearing was 8% at C7, 28% at XIPH and 47 % at ASIS level (Bates and Hanson 1996, Figure 3).

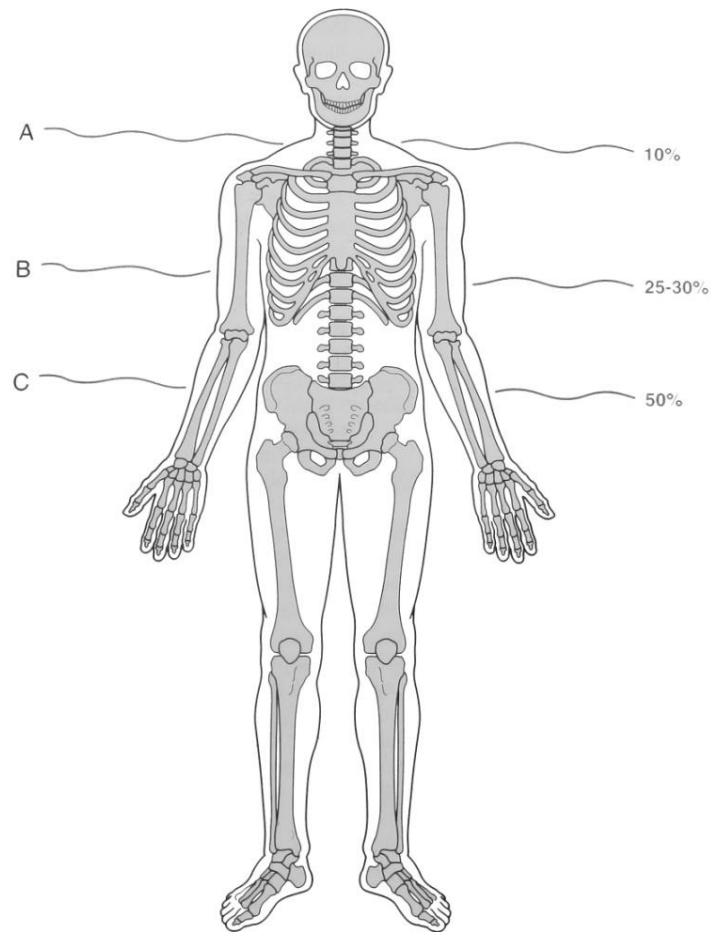


FIGURE 3. Percentage of body weight bearing: A: C7, B: XIPH, C: ASIS level (Bates & Hanson, 1996).

2.1.3 Physiological changes during thermoneutral water immersion

The physiology of immersion is a well-studied and understood phenomenon (Pendergast et al. 2015). Immediate and delayed responses can be recorded as cardiorespiratory, endocrine and renal responses. Thermoneutral water (34 or 35 °C for 3-6 hours) responses mainly depend on the level of immersion. As the previous section describes, increased water level lead to higher water pressure exerted on the body. Pressure causes fluid shift from the lower region to less pressured areas of the body which leads to several physiological changes all over the human body (Pendergast et al. 2015).

Majority of aquatic studies focuses on head-out water immersion which exerts much more water pressure on the body than the depth at ASIS level (Hall et al. 1990; Pendergast et al. 2015; Boussuges 2006). The following section gives general idea about the physiological changes during standing and/or sitting water immersion.

During seated or upright position in thermoneutral water, blood shift/translocation (approximately 700 ml) causes an increase in blood return to the heart by compressing the venous compartment (Hall et al. 1990; Pendergast et al. 2015). Increased blood return is the basis for all the physiological changes associated with immersion.

Heart rate (HR) changes are dependent on the form of immersion. During immediate head out immersion it decreases, while during slow, step-by-step immersion it is slightly increased first, before it tends to decrease compared to dry land condition (Schega et al. 2007). Overall, HR tends to decrease if the body is immersed to thermoneutral or cold (26-27°C) water (Löllgen et al. 1981; Mourot et al. 2008). However there are studies that found no change in HR during thermoneutral water immersion at both gender (Boussuges 2006).

The amount of blood ejected from the left ventricle (stroke volume - SV) is increased as a result of the increased cardiac pre-load during immersion (Park et al. 1999). The same study also found that SV tends to be even greater in colder water (30°C) than in thermoneutral water. Schega et al. (2007) reported that SV decreased during stepwise immersion but

increased during complete immersion. The effect of water to increase SV has been recorded after 10 minutes from the start of the immersion (Boussuges 2006). The increase of SV is much greater in navel level during standing position compared to chest and neck level immersion (Itoh et al. 2007).

Cardiac output (function of stroke volume and heart rate) has shown to increase during water immersion. However, studies report different level of increase (from 12% to 50% compared to dry land control condition), it shows steady increase already at the hip level (Löllgen et al. 1981; Hall et al. 1990; Boussuges 2006; Park et al. 1999). Schega and colleagues (2007) found that during stepwise immersion the cardiac output decreases.

During head-out water immersion not just cardiovascular changes occur, respiratory system is also affected, including vital capacity, total lung capacity, functional residual capacity, and expiratory reserve volume (Hall et al. 1990; Dahlbäck & Jönsson 1978). The aforementioned lung volumes tend to decrease during immersion. Hydrostatic pressure translocates blood from the lower region of the body to the chest which increases the demands of the respiratory system (Pendergast et al. 2015). Blood redistribution decreases lung compliance which could also decrease lung volumes (Dahlbäck & Jönsson 1978).

The energy cost of breathing is also changed during immersion (Pendergast et al. 2015). During immersion the energy cost of breathing is increased as a result of increased airway resistance and a decrease in the net mechanical efficiency of the respiratory muscles (Pendergast et al. 2015; Held & Pendergast 2013).

2.1.4 Water as an exercise medium – aquatic therapy

As mentioned in the previous section water provides a unique medium for physical therapy. The following section briefly mentions the different areas where aquatic therapy can be useful. The importance of this section is to see how widely aquatic therapy has been used, yet there is a lack of neuromuscular understanding behind the different methods.

Water-based exercises can be divided based on the purpose of the training. Aquatic fitness training is carried out in water and the main aim is to maintain or improve physical fitness with the help of different methods and equipment. The main goal of the aquatic training is to improve the athletes' physical performance (Pendergast et al. 2015). One can find excellent ideas to alter aquatic therapy to different body regions and sports-specific modulations from Thein and Brody (1998). Also different applications for cardiopulmonary, respiratory, geriatric, osteoporosis rehabilitation and for pregnancy also exist (Becker 2009). Due to the decreased gravitational forces, the aquatic environment provides a safe opportunity for body weight controlled training. Cold-water immersion or contrast water therapy is also beneficial for athletes for faster recovery (Versey et al. 2013).

Aquatic therapy/rehabilitation is “a combination of water based activities aimed to total or partial orthopedic and neurological rehabilitation” (Pendergast et al. 2015). Sport-specific exercises and core strengthening can be easily applied to aquatic therapy for faster rehabilitation or to vary land-based exercise programs (Thein & Brody 1998). Moreover the safe environment of water can be used for early physical therapy after orthopedic surgery (Villalta & Peiris 2013). In their review paper Villalta et al. (2013), found that there is no increased infection risk of early aquatic therapy, as long as the surgical site is covered with an appropriate waterproof dressing.

Osteoarthritis (OA) is one of the leading causes of decreased function and quality of life and aquatic therapy has been widely used as a prevention and treatment (Waller et al. 2013). Foley et al. (2003) showed that comparing land-based gym exercises with aquatic therapy the functional gains were similar in elderly subjects with hip or knee OA. Similar study design showed also improved physical functions, strength and quality of life even after 6 weeks of follow-up (Hinman et al. 2007).

Wang et al. (2007) found no pain relief after 12 weeks of aquatic therapy with hip or knee OA patients. In the intervention group the flexibility, leg muscle strength and aerobic fitness improved, however in walking distance no further improvement was recorded after 6 weeks compared to 12 weeks. Acute effects of aquatic treadmill walking positively affected the

gait kinematics compared to dry-land treadmill walking in 14 participants with OA (Roper et al. 2013). The perceived pain was 100% higher after land than aquatic exercise.

Further meta-analyses showed that aquatic therapy has moderate beneficial effects on pain, physical function and quality of life in adult subjects with OA (Barker et al. 2014; Waller et al. 2014). It has been mentioned by both research groups that there is a lack of studies that follow-up the long-term effect differences between land-based and aquatic therapies. A systematic review from Al-Qubaeissy and colleagues (2013) suggests that hydrotherapy treatment has a positive role in reducing pain and improving health status of patients with rheumatoid arthritis.

During a controlled clinical trial Baena-Beato et al.(2013) used high frequency (five times per week) aquatic therapy to treat chronic low back pain. In the 24 subjects during the two months intervention, the level of low back pain decreased, quality of life increased and body composition improved (increased lean muscle mass, decreased total body fat) compared to the control group.

Aquatic therapy has also been used to improve the mobility of adults with neurological diseases (Marinho-Buzelli et al. 2015). In this systematic review the researchers found that the therapy has “fair” increase in dynamic balance and gait speed in adults with stroke, multiple sclerosis, Parkinson’s disease, spinal cord injury, spastic paresis or brain injury. The authors didn’t find strong evidence to suggest that aquatic therapy is superior to land therapy in mobility recovery.

2.2 The Hoffmann reflex

The Hoffmann reflex (H-reflex) is one of the most studied reflex and allows to study the sensimotor integration and plasticity of the central of the central nervous system (Knikou 2008). First described by Hoffmann (1918), it is the result of excitation of α -motoneurons (Knikou 2008). The percutaneous electrical stimulation that evokes the H-reflex bypasses the

muscle spindle and involves both afferent sensory and efferent motor arcs (Knikou, 2008; Zehr, 2002, Figure 4).

Electrical stimulation of the nerve above the threshold of activation of the Ia afferents and α -motoneuron pool will produce two responses, a short-latency M-wave (direct motor response) and the H-reflex (Zehr 2002). The H-reflex and M-wave do not recruit the same α -motoneurons (Knikou 2008). By incrementally increasing the electrical stimulation, H-max can be reached, which represents the fullest extent of reflex activation and at higher stimulation level, maximal muscle activation can be reached (M-max, Figure 5). After a certain stimulation intensity H-reflex amplitude decreases due to the collision of the antidromic volley with the orthodromic afferent volley (Knikou 2008). Changes in H-reflex amplitude depends on the motoneuron excitability and the ongoing presynaptic inhibition of Ia fibres (Knikou 2008). By normalizing the H-reflex to the maximal M-wave allows between subject and condition comparison (Zehr 2002). The most studied muscle with H-reflex is the soleus due to its importance in human gait and accessibility during rest (Pierrot-Deseilligny & Mazevet 2000).

The H-reflex method has been used to its simplicity and its adaptability for the current requirement. As the study is an early attempt to record the prolonged effect of immersion it is probably will serve as a guide for future studies where more precise mechanisms can be addressed. Another property of the H-reflex recording is that it is the only method that allows various motor task executions during testing (Pierrot-Deseilligny & David Burke 2012) and the current findings can serve as reference for future protocols that include motor tasks.

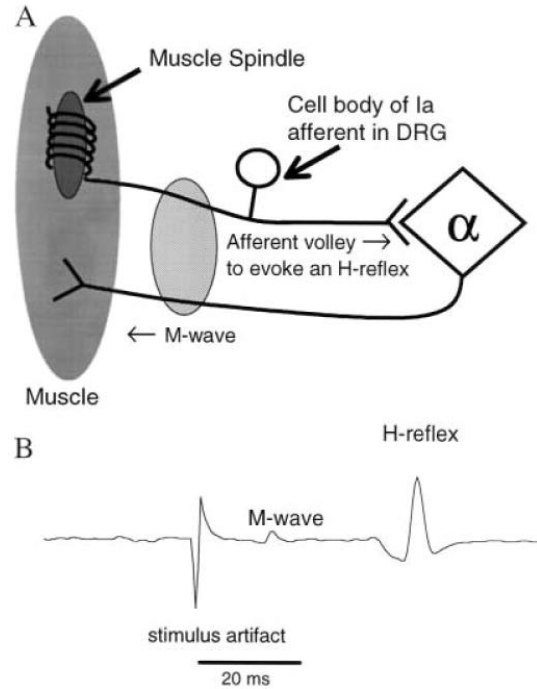


FIGURE 4. A: Schematic illustration of the spinal processing of the monosynaptic component of the H-reflex. Grey ellipse indicating the electrical stimulation of the mixed peripheral nerve. B: Stimulus-triggered H-reflex and M-wave during voluntary contraction (10% of MVC) (DRG – Dorsal Root Ganglion). (Zehr, 2002)

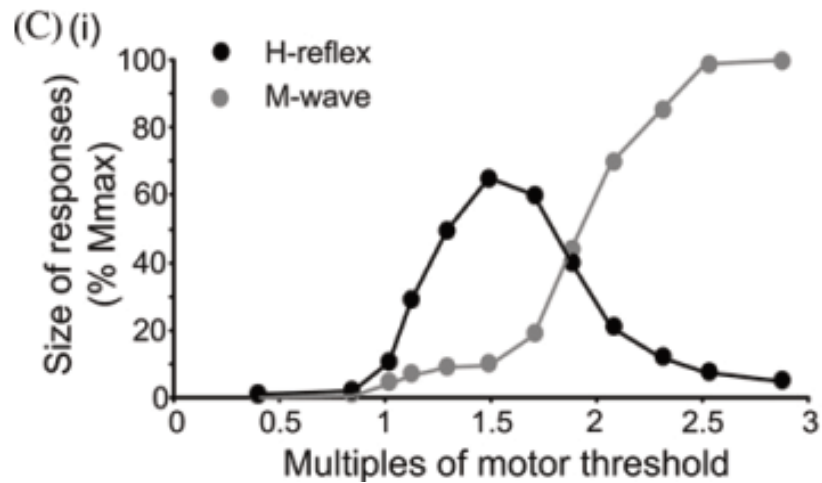


FIGURE 5. “Typical” H-reflex recruitment curve of the soleus muscle in standing position (Knikou, 2008)

2.2.1 General experimental considerations for H-reflex during immersion

Since the H-reflex is really sensitive and can be highly altered, it requires caution when the data is interpreted. The following section discusses and includes the variables that should be considered during aquatic therapy.

As the aquatic therapy can be used for both sexes without age restriction it is important to compare the neuromuscular responses along genders and age groups. During rest there is no gender difference in maximal H-reflex, M-wave and maximal H/M ratio (Buschbacher 1999; Christie et al. 2004; Solianik et al. 2014). Cheng et al., (2007) reported sexual difference in spinal excitability modulation during bipedal locomotion observation. As the subjects were asked to observe specific ankle positions (heel, stand and toe position) on a computer screen, female participants showed higher modulation of H-reflex of the soleus muscle (increase of maximal H-reflex amplitude). The findings of Cheng et al. (2007) suggests that females can modulate spinal excitability in higher amplitude, however no further evidence supports this findings. In practical sense it may be possible for women to modulate their reflex responses during immersion to a greater degree.

Nerve degeneration by increasing age could be the possible mechanisms why older individuals have longer reflex latency (slower nerve conduction velocity) (Buschbacher 1999; Dewhurst et al. 2005), smaller reflex amplitude (Buschbacher 1999; Huang et al. 2009; Dewhurst et al. 2005) and smaller maximal H/M ratio (Kido et al. 2004; Baudry & Duchateau 2012) compared to young individuals. Elderly subjects also showed slower H-reflex facilitation in the soleus muscle before voluntary movement (Burke & Kamen 1995), lower degree of reflex modulation between various posture positions (Koceja et al. 1995; Chalmers & Knutzen 2002) compared to young adults. The results suggest that elderly subjects are slower to adapt or react to gait related changes which can lead to higher risk of falling. Beneficial effect of the aquatic environment can be useful in older population as provides safer environment. Moreover Earles et al. (2001) indicates different motoneuron pool control – lower presynaptic inhibition gain - in elderly subjects during voluntary contraction (10% and 20% of MVC), while the presynaptic inhibition was significantly greater in young adults

at rest. On the other hand, maximal M-wave is not altered by age (Chalmers & Knutzen 2002; Dewhurst et al. 2005), which can support the idea to use this parameter to normalize the reflex parameters to compare different groups (Zehr 2002).

As previously mentioned water immersion at different level decreases the weight bearing on the body (Bates & Hanson 1996). Tsuruike et al. (2012) reported stable maximal M-wave in different weight bearing conditions, however maximal H/M ratio altered in both elderly and young adults. Maximal H/M ratio increased significantly when 50% of body weight applied on the elderly subjects but it decreased in young group (Figure 6). It is possible that elderly individuals have better benefits during aquatic training as the decreased maximal H/M ratio reflects greater reflex excitability (Tsuruike et al. 2012). Decreased weight bearing and supportive feeling of the water could also help to avoid sway dependent modulation of H-reflex (Tokuno et al. 2007), and to avoid suppression of soleus H-reflex during unilateral stance – as part of movement therapy (Huang et al. 2009).

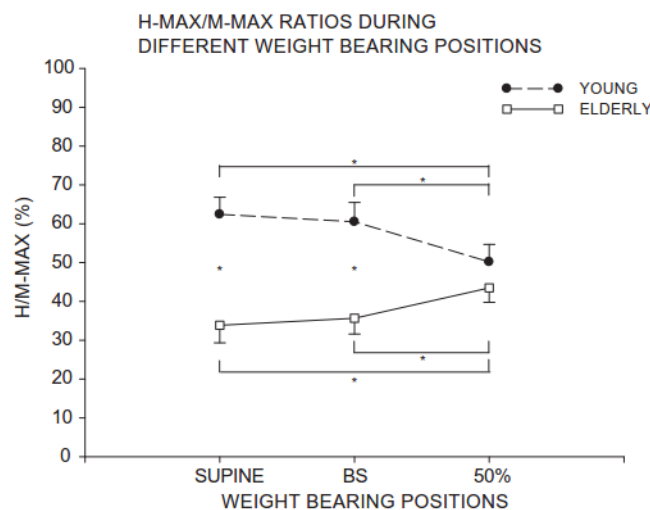


FIGURE 6. Alteration of maxima H/M ratio in different weight bearing conditions in young and elderly subjects (Tsuruike et al., 2012)

Wide-range of therapeutic solutions and floatation devices can be used to provide supportive, assistive or resistive forces during aquatic therapy (Pöyhönen et al. 2002). The higher resistance requires higher force output which can enhance the reflex modulation during immersion. Soleus maximal H/M ratio is increased during isometric contraction (Butler et al.

1993), concentric contraction and eccentric contraction (Kallio et al. 2010; Hultborn et al. 1996; Romanò & Schieppati 1987; Klass et al. 2011) compared to resting condition. Kallio et al. (2010) have shown that increased maximal H/M ratio is present at both young and elderly males, with the lowest maximal H/M ratio recorded at eccentric phase and highest at concentric contraction, 40% of maximal voluntary contraction level (Figure 7).

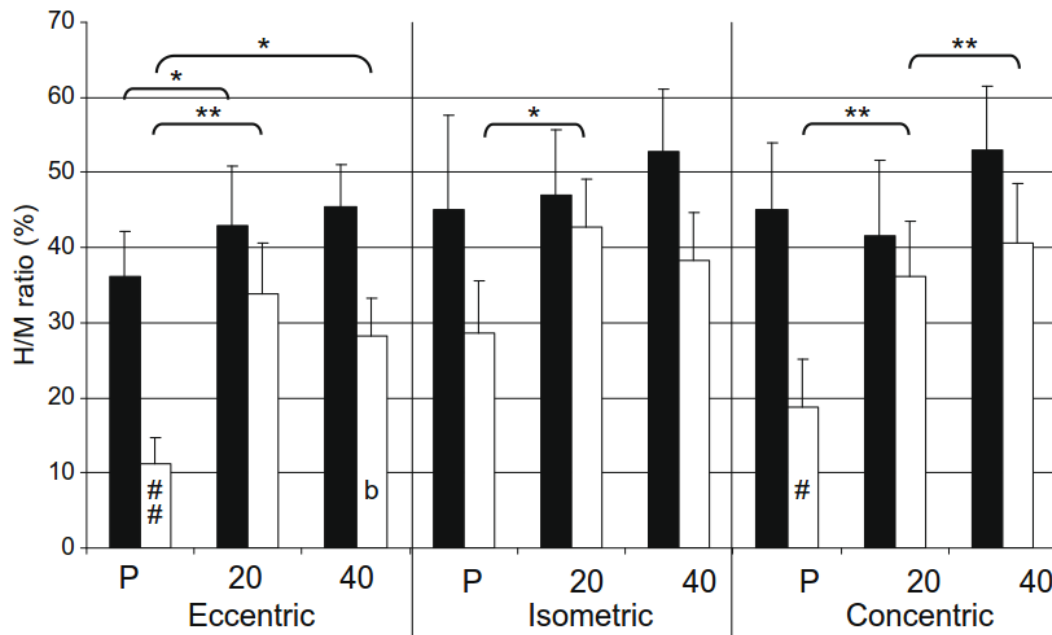


FIGURE 7. Changes in mean H/M ratio of young (black) and old (white) individuals during passive (P), 20% and 40% activation (Kallio et al. 2010).

Several electromyographic study analysed muscle activation of different exercises in water (Masumoto et al. 2004; Masumoto & Mercer 2008; Kelly et al. 2000). These findings shows decreased muscle activity when it was assessed by integrated EMG activity of the muscle. On the other hand, metabolic cost of underwater walking and jogging is higher in water (Gleim & Nicholas 1989; Di Prampero 1986). Higher force output is required to overcome the more dense environment in water which can increase spinal excitability compared to dry land even when the same set of movement is executed.

Apart from its protective properties, the water could give stimulation by altering balance with the changing directions of water flow. This property of the water can be used for training purposes and it can be especially useful for elderly population. Trimble and Kocejka (1994) recorded H-reflex reduction during and after balance training as adaptation of the nervous system, while maximal M-wave remained stable. Reduction of maximal H/M ratio was required to avoid perturbation. These results has been supported later by short training (3 days) (Mynark 2005) and long training (4 weeks of balance training) protocols (Taube et al. 2007). Effect of balance training and it's part in aquatic environment can be useful in both young and elderly subjects as the modulation of maximal H/M ratio (decrease of maximal H/M ratio) appeared in both groups (Mynark & Kocejka 2002, Figure 8).

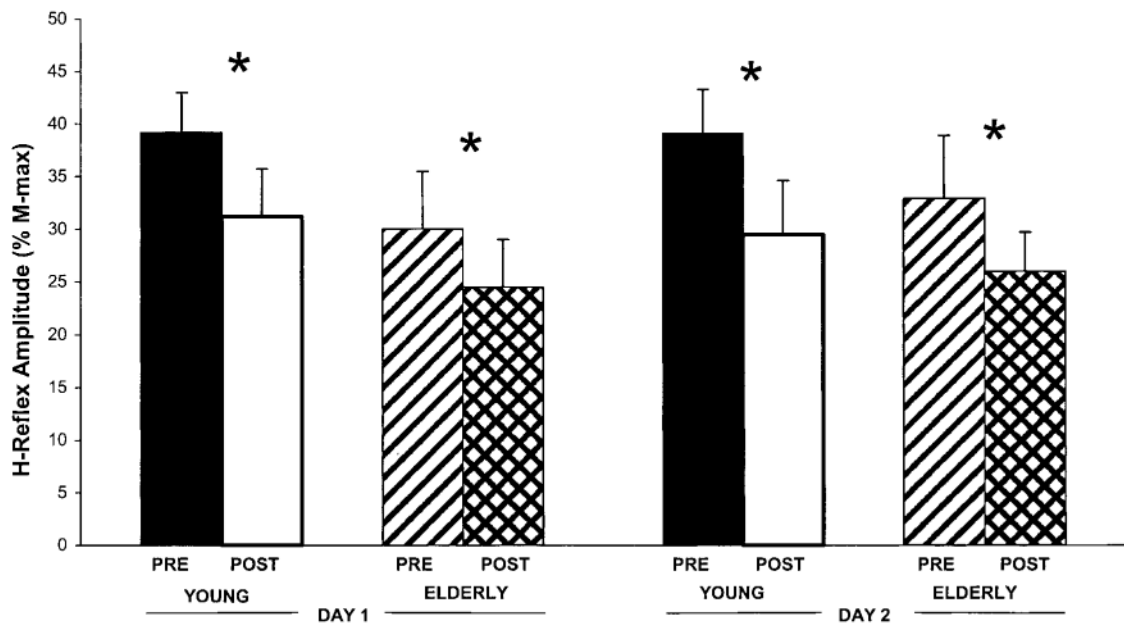


FIGURE 8. Downregulation of soleus H-reflex after balance training in young and elderly subjects (Mynark & Kocejka, 2002).

Appropriately selected water temperature could also enhance reflex response during therapy session. Krause et al. (2000) showed inverse correlation between ankle-skin temperature and H-reflex amplitude (current intensity at 75% of maximal H-reflex). By decreasing the ankle-skin temperature the H-reflex increased ($r = -0.95$), and during rewarming phase the H-reflex showed further increase ($r = 0.74$) (Figure 9). Similar finding has been found by Solianik et al., (2014) where increased maximal H-reflex and maximal H/M ratio was recorded after

cooling protocol. Maximal M-wave showed no change. The results suggest that reflex excitability changes can be enhanced by lower water temperature.

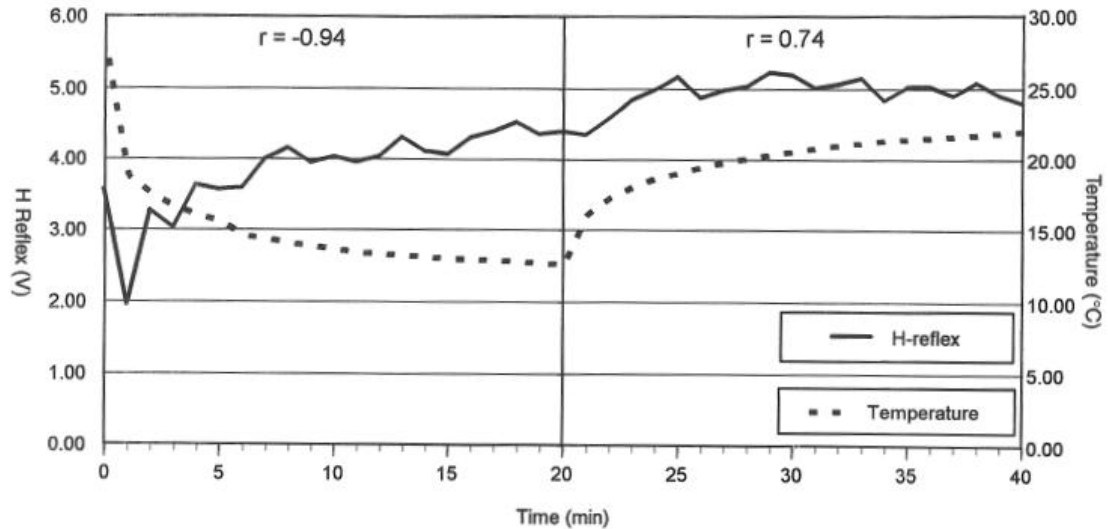


FIGURE 9. Changes of the H-reflex amplitude during cooling and rewarming protocol (Krause et al. 2000).

As mentioned before increasing the water depth, the hydrostatic pressure increases over the surface of the body (Brody & Geigle 2009). Massage study from Goldberg et al. (1992), applied different pressure on the skin surface of the calf (deep massage – 2.5 kPa, light massage - kPa) showed decreased maximal H-reflex amplitude but unchanged maximal M-wave of the soleus in both condition. Decreased H-reflex amplitude has been recorded in similar protocol design even when sensation feeling has been abolished by anesthetic (Morelli et al. 1999). However, the comparison of the results should be taken carefully as the massage only applied to the small portion of the skin surface, while during immersion the whole skin area is under pressure. On the other hand, the results of Morelli et al. (1999) suggest that deep mechanoreceptors can be activated in various conditions that can be useful for therapeutic applications – early post-operative therapy with anesthetics can benefit from immersion.

As the water provides multiple stimulation at the same time the distinguish of different factors and their effect on the neuromuscular system is hard. Up to date we found no study that controlled immersion in multiple level like depth, temperature, elapsed time, water flow or body position. We assume that the high level of control of the above mentioned factors could help to understand the effect of immersion on the neuromuscular system, recorded by the Hoffmann reflex and M-wave.

2.2.2 Reliability of the Hoffmann reflex and M-wave

Within the thousands of studies that used H-reflex, around only 20 studies main aim was to establish the reliability of the reflex (Hayes et al. 2009, Table 1). The most studied muscle in reliability studies is the soleus, probably because its easy accessibility and the most measured parameter is the maximal H-reflex, M-wave and the maximal H/M ratio. Test-retest reliability varies from 0.29 (Ali & Sabbahi 2001) to 0.99 (Palmieri et al., 2004; Stowe et al. 2008) during rest in prone and sitting position. The reliability, however smaller during higher voluntary activation (Chen et al. 2010) or higher joint angle (Alrowayeh & Sabbahi 2006). Mynark (2005) showed variety of H-reflex amplitude within session and recommended to use 5 to 10 averaged recordings for H-reflex studies. The longest reliability study recorded maximal H/M ratio over 3 years and reported 5% variability in five subjects (Nielsen et al. 1993). The different reliability values could be the result of different protocol set-up or recording error.

Maximal H-reflex amplitude and maximal H/M ratio shows alteration due to changed joint position and for voluntary activation. Frigon and colleagues (2007) showed significantly smaller maximal H-reflex amplitude and maximal H/M ratio during plantar- and dorsiflexion. On the other hand, posture related changes in H-reflex parameters show contradictory results in the literature. While some authors report increases and decreases within their subjects (Kocejka et al. 1995), recent studies show a decrease in maximal H/M ratio during standing compared to sitting (Kawashima et al. 2003; Tokuno et al. 2007) or to lying position (Shimba et al. 2010).

Maximal M-wave remains unchanged between standing and supine position (Koceja et al. 1995), different weight bearing conditions (Tsuruike et al. 2012), during balancing exercise (Trimble & Koceja 1994), during passive shortening action (Pinniger et al. 2001) and after 12 weeks of resistance training (Aagaard et al. 2002). Changes of maximal M-wave under area EMG were recorded when the ankle joint angle has been changed: M-wave is larger during dorsiflexion compared to plantarflexion and plantarflexion with 40% (Tucker & Türker 2007).

Although H-reflex reliability has been assessed in detail, a limitation of previous studies in relation to this thesis is that they were all conducted on land. To date, no such study has been conducted using H-reflex testing during immersion. This information is important because it would give an indication of the suitability of this method for aquatic applications.

2.2.3 Neuromuscular changes during water immersion

The effect of water immersion on reflex parameters has been studied only in a few papers until today. The study from Pöyhönen & Avela (2002b) is one of the few studies that conducted underwater measurement and it also inspired our recent study. Overall, three subject's data were analyzed, whom immersed until mid-sternum level in 30°C water. Maximal H/M ratio of the soleus, maximal isometric plantar flexion (MVC) and tendon tap reflex were recorded on dry and in water immersed conditions. Results showed decreased H-reflex during immersion but no changes in maximal M-wave (31.0 ± 27.0% decrease in maximal H/M ratio, Figure 10). There was a significant reduction in maximal isometric force (1064 ± 226 vs. 919 ± 192 N) and in the aEMG/force ratio during contraction (-21.5 ± 8.7% vs. -24.2 ± 0.10%). The authors suggest that the impairment of neuromuscular function is due to reduced function of some reflex mechanisms which is caused by the hydrostatic pressure and/or reduced gravitational conditions. However, they mention the relevant effect of presynaptic inhibition of Ia afferent terminals and disfacilitation of the α -motoneuron pool.

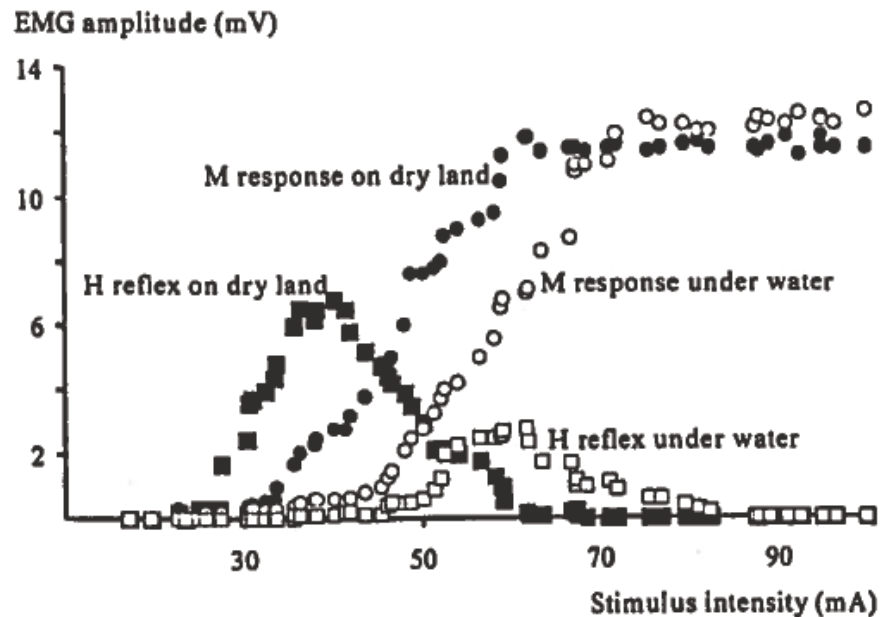


FIGURE 10. Changes of H-reflex and M response during water immersion compared to dry land condition. (Pöyhönen & Avela, 2002).

Cronin et al. (2016) recently showed that the maximal H/M ratio increases after 5 minutes of water immersion. 7 hyperreflexive and 7 age-matched healthy people participated in that study. Results showed acute increase in maximal H/M ratio after immersion, which was sustained in healthy subjects, while in the patient group the ratio decreased close to control value. After 5 minutes of land rest, maximal H/M ratio decreased non-significantly below the control value (Figure 11). As the authors highlights in their discussion there are multiple factors that could lead to the different findings between this and Pöyhönen's above mentioned study (Pöyhönen & Avela 2002). Cronin et al. (2016) used waterproof trousers, which could alter the sensation of immersion, while Pöyhönen and Avela (2002b) used compressive joint support that could modulated proprioception during testing.

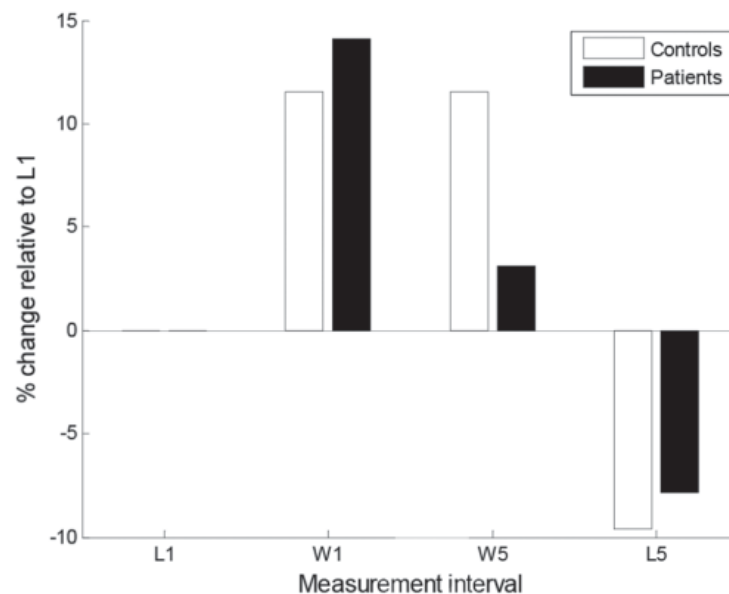


FIGURE 11. Changes of maximal H/M ratio in healthy and hemiplegic group due to water immersion (Cronin et al., 2016).

Sato et al. (2012) found that femur-level water immersion in sitting position can result in increased activity in both sensory and motor areas of the cortex, measured by functional near-infrared spectroscopy (fNIRS). Immersion of the leg in 34°C water lead to an increase in oxygenated hemoglobin concentration in the primary somatosensory area (S1), parietal association area (PAA), supplementary motor area (SMA) and primary motor area (M1). Most interestingly the significant changes occurred already at 20 s in S1, 40 s in PAA and

100 s in SMA and M1 after onset of water immersion. Oxygenated hemoglobin correlated with brain electrical activity and the authors claim it reflects changes in the cortical activity and metabolism. Results suggest that the water immersion could enhance motor learning during rehabilitation.

The effect of two different water stimulation conditions on motor-evoked potentials (MEPs) in the right first dorsal interosseous muscle has been studied recently by Sato et al. (2014). Whole-body immersion (WI) and whole-body water immersion with water flow (WF) conditions' effect was assessed after 15-minute-long intervention from 8 healthy subjects. After intervention the TMS experimental sequence was repeated 3 times every 10 minutes. Resting motor threshold (rMT) decreased after intervention but active motor threshold (aMT) remained stable. Only WF condition increased motor-evoked potential (MEP) amplitude after the first 30 minutes of intervention, while short intracortical inhibition (SICI) decreased. The results suggest that whole-body water-flow condition modulates corticospinal excitability and short intracortical inhibition, but not intracortical facilitation.

2.3 Ultrasonography during immersion

Muscle ultrasound is an excellent and relatively cheap technique to visualize and study muscle tissue in-vivo (Pillen & Pillen 2011). Using ultrasound (US) it is possible to calculate the recorded muscle thickness, fascicle length and pennation angle during rest or during movement (Thoirs and English 2009; Ohata et al. 2006; Fukunaga et al. 1987, Figure 12). Reliability of the recording highly depends on human factor, and high experience is required for clinical recordings (Chew et al. 2008).

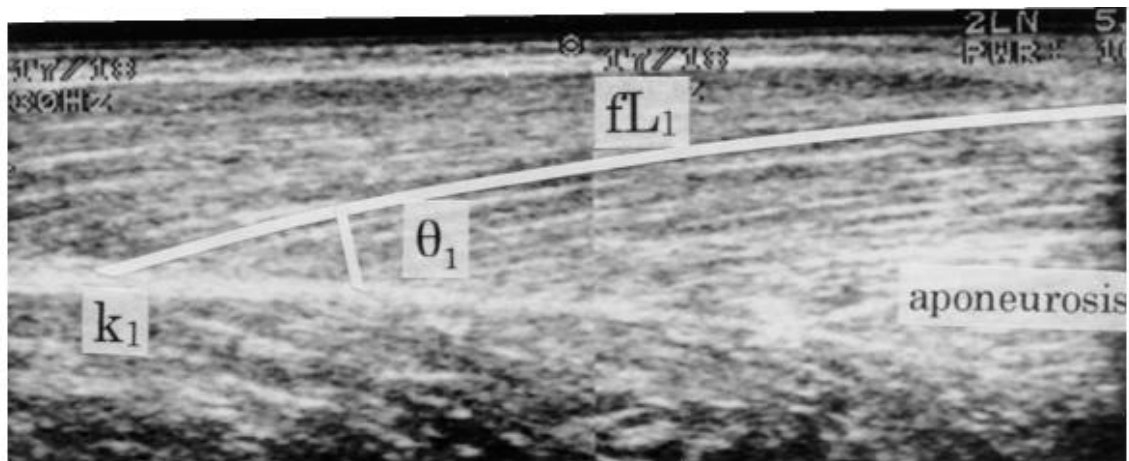


FIGURE 12. Ultrasonic longitudinal image of Vastus lateralis muscle. “Fascicle length (fL) was determined as length of a line drawn along ultrasonic echo parallel to fascicle. Fascicle angle (θ) was determined as angle between echoes obtained from fascicles and deep aponeurosis in ultrasonic image” (Fukunaga et al. 1987)

Soleus fiber length and pennation angle shows significant changes in different ankle positions and voluntary effort, while the thickness shows no changes in different ankle positions (Maganaris et al. 1998; Kawakami et al. 1998). In the literature, no study has been found that assess muscular changes during water immersion via ultrasound recording.

3 PURPOSE OF THE STUDY

As seen from the previous review of literature, neuromuscular changes during water immersion are poorly understood (Pöyhönen & Avela 2002; Cronin et al. 2016). Therefore, the main aim of the study was to determine and design a reproducible protocol to assess underlying neuromuscular and muscle architectural changes during prolonged water immersion, using H-reflex and ultrasound recording.

The following hypotheses were formulated:

- I. During 1-hour long water immersion maximal H/M ratio increases as the peripheral reflex excitability increase.
- II. During 1-hour long water immersion no change in muscle architecture occur.
- III. Maximal H/M ratio, maximal M-wave and maximal H-reflex peak-to-peak amplitude remains the same during 1-hour dry land condition.
- IV. Maximal H/M ratio, maximal M-wave and maximal H-reflex peak-to-peak amplitude values shows high repeatability after one week on both dry land and during water immersion.

4 MATERIALS AND METHODS

4.1 Subjects

A total of 12 volunteers participated in the study (age: 24 ± 3 years; height: 175 ± 6 cm; body mass: 69 ± 8 kg). All subjects were male, physically active students from the university. Due to the physical parameters of the seat, an inclusion criterion for height was between 165-185 cm. Previous studies reported sex differences in spinal excitability during observation of bipedal locomotion (Cheng et al. 2007). In order to avoid false interpretation from the data only male subjects were recruited. None of the subjects had any history of neurologic disease. All subjects provided written informed consent, and the study was approved by the local ethics committee. Subjects were asked not to perform any strenuous physical activity for 24 hours before data collection. Caffeine intake was restricted two hours prior to testing (Eke-Okolo 1979; Walton et al. 2003; Kalmar & Cafarelli 1999). To avoid possible influence of circadian rhythm, each subject was required to come to the experimental session at the same time of day. With one subject the recording couldn't be finished in water immersed condition on the second recording day, however the rest of his data were still included in the analysis.

4.2 Protocol and measurements

One test session included a total of 11 recruitment curves – one high resolution curve and 10 (5 dry land and 5 water immersed) fast recruitment curves (Figure 13). Each session started with the preparation of the electrode sites and the adjustment of the apparatus for the most comfortable position. The location of the electrodes and the chair position was marked with permanent marker on the first day and the subjects were asked to redraw the markers between the two testing days. The test session was repeated after 7 days (first test session: Day0; second test session: Day7).

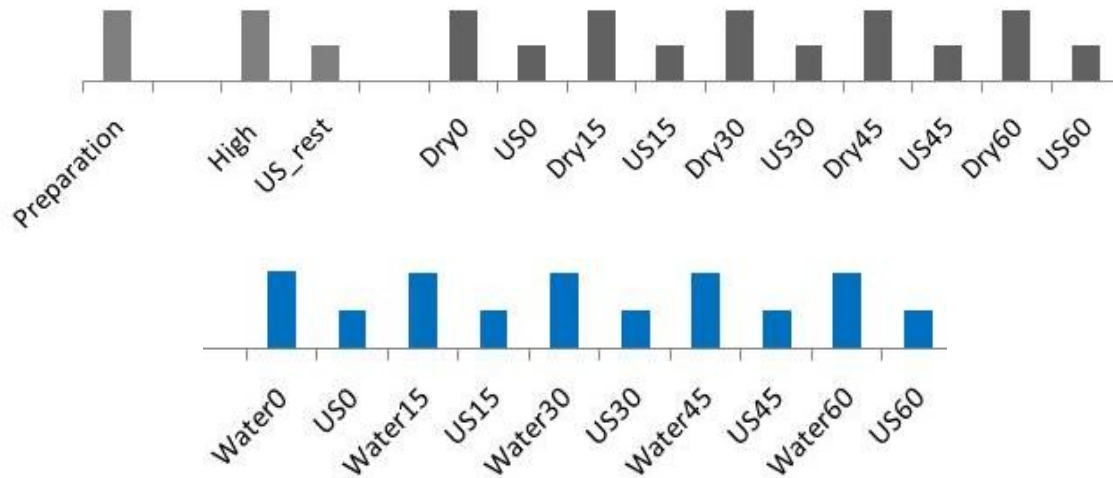


FIGURE 13. Protocol set-up for the thesis (High – Reference H-reflex curve; US – Ultrasound recording of the muscle thickness; US_rest – Reference muscle thickness recording; Dry- H-reflex recording on dry land; Water – H-reflex recording during immersion; numbers indicate the elapsed time in the given condition; note that the ultrasound recording was made immediately after the H-reflex curve recording).

Apparatus

A custom-made water tank was built by the department's technical staff to simulate aquatic conditions. The 160 cm long, 80 cm high and 70 cm wide tank was big enough for all subjects to fit in comfortably. One of the side walls was made from transparent plastic to allow observation. The tank was filled with tap water. In order to keep the water clean it was replaced every week, and during that time a commercial water cleaning system was used (Cristalprofi e1501, JBL, Germany, Neuhofen,). Water temperature during the testing was maintained at thermoneutral, 34 °C (Pendergast et al. 2015, 3619 Aquarium Heater, EHEIM, UK, Edmunds).

Subjects were seated in a custom-made chair (Figure 14). The design of the chair kept the hip and knee angle constant at 90° and 150°, respectively, while the ankle remained fully rested. During pilot testing when the ankle was fixed to 90°, in some cases it resulted in loss of sensation in the leg and changes in the recruitment curve – namely the H-reflex response disappeared. For this reason the subjects were instructed to keep the ankle in the most comfortable position, even though it resulted in differences in muscle length between subjects (Gerilovsky et al. 1989) and thus different ankle joint angles between subjects. However, the same position was kept within day, condition and between days for each subject. The length

of the lower part of the chair was adjustable to accommodate different leg lengths. This length was marked for each subject to enable consistent leg position between tests. Head position was controlled by asking subjects to focus on a symbol placed 1.5 meters in front of him, both on dry land and during immersion.



FIGURE 14. Custom-made chair for H-reflex recording on dry land and during immersion.

The level of immersion was approximately 20 cm, which equals to 15 mmHg of water pressure on the muscle (Ohanian & Markert 2007, Figure 15).

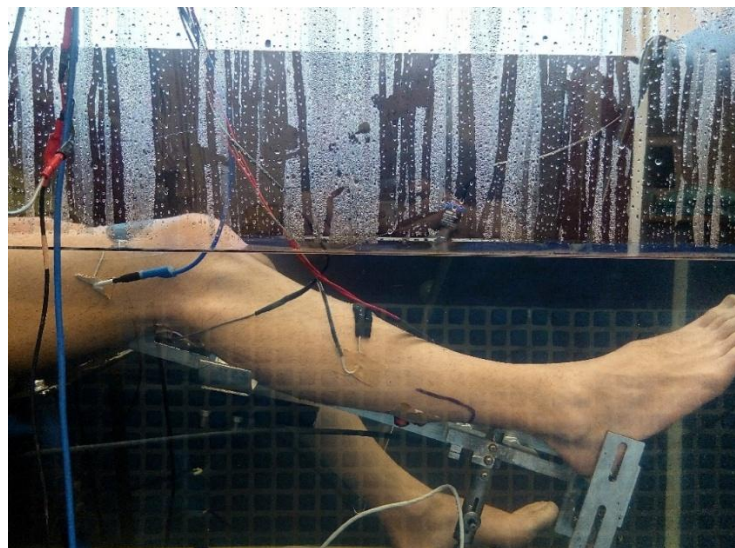


FIGURE 15. Example image of an immersed lower leg during the test session.

Measurements

Electromyogram (EMG) signals from the soleus muscle were recorded using an Eisa 16-2 EMG system. Oval-shaped, bipolar, pre-gelled silver chloride (Ag/AgCl) self-adhesive surface electrodes (AmbuBlueSensor N, Denmark, Ballerup) were positioned along the muscle fibers between the center of the innervation zone and the distal tendon of each muscle. The inter-electrode distance was 2 cm. To keep the inter-electrode resistance low ($<2\text{k}\Omega$), the skin was shaved, rubbed with sandpaper, and cleaned with 60% alcohol. Data were sampled at 1000 Hz (gain 500) using an A/D converter (CED 1401, Cambridge Electronics Design, Cambridge, UK) and Spike2 software (version 6.17, Cambridge Electronics Design, Cambridge, UK).

To waterproof the EMG setup, the electrode connectors were covered in moldable, water resistant silicone. Button electrode connection areas were also covered in water resistant insulating tape. Based on extensive pilot testing, this method did not have any adverse effects on signal quality. The electrodes on the skin surface were covered with waterproof tape (OpsiteFlexigrid 15x20 cm; Figure 16). This method was used for the EMG and stimulation electrodes. Previous studies recommend a similar method for underwater EMG recordings (Silvers & Dolny 2011).



FIGURE 16. Insulation of the anode electrode with Opsite water-resistant tape.

Every additional cable connection was secured with the same moldable silicone and insulating tape (Figure 17). Raw EMG signals were regularly monitored to ensure that water leakage did not occur during immersion (Kalpakcioglu et al. 2009; Cuesta-Vargas & Cano-Herrera 2014).

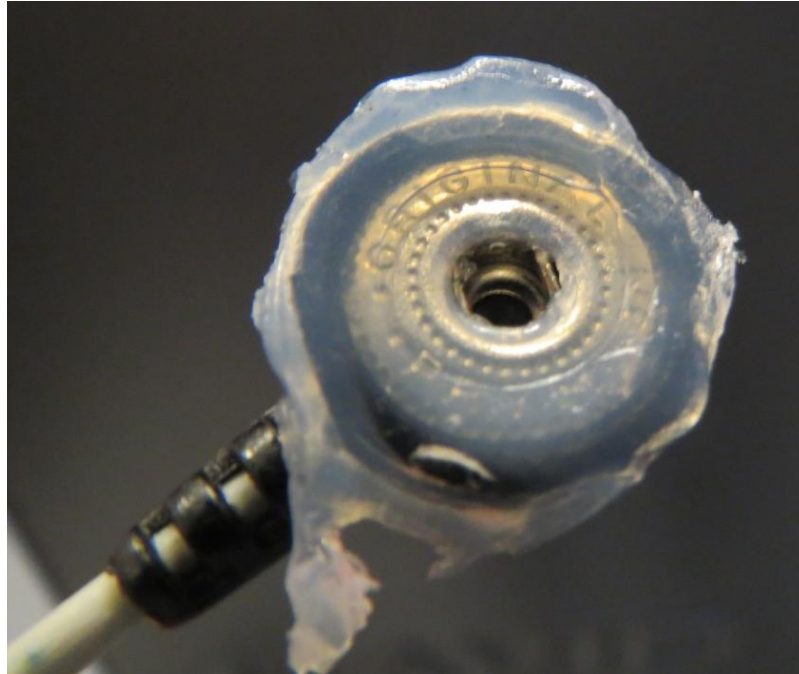


FIGURE 17. Electrode insulation with moldable, waterproof silicone.

The H-reflex and M-wave were elicited via electrical stimulation of the tibial nerve in the right leg. After determining the optimal stimulating location, a cathode electrode (AmbuWhiteSensor 4500M) was placed over the tibial nerve in the popliteal fossa, and the anode (5 x 8 cm) was positioned superior to the patella. Stimulation intensity was delivered by a linear isolated stimulator (Stimsol; Biopac Systems Inc; California, USA). For reflex recruitment curve recording, Spike2 software was used. The sequencer function of the program allowed predetermined intensities and interstimulus intervals to be used.

A High Resolution Curve was recorded in order to set the additional stimulus intensities for the actual test (Figure 18). Starting from 0 mA current, the intensity was increased by 0.5 mA every 8-12 seconds (0.125 - 0.083 Hz). A random interstimulus interval (ISI) was used in order to avoid anticipation (Ivanchenko 2014). The test was stopped after the maximal

muscle response (M_{\max}) was achieved, defined as no further increase in amplitude at 5 constitutive intensities. Each high resolution recruitment curve test lasted approximately 10-12 minutes.

H-reflex and M-wave threshold-intensity were extracted from the high resolution curve. Thresholds were determined by the apparent difference from baseline. $H_{50\%desc}$ was the closest stimulation intensity for the 50% of maximal H-reflex amplitude. With these reference points a fast test recruitment curve was calculated using a manually written script that contained (Brinkworth et al. 2007; Knikou & Rymer, 2002, Figure 19):

- 14 equally distributed stimuli between 80% of H_{th} and 110% of $H_{50\%desc}$;
- 8 equally distributed stimuli between M_{th} and 110% of M_{\max} ;
- One supramaximal stimulus at 150% M_{\max} .

Based on the findings of Cronin et al. (2016), the neurophysiological changes during immersion appears almost immediately. In order to record acute changes in the H-reflex parameters relatively short and fast recording was needed. Comparison of the high resolution curve with the fast test recruitment curve showed identical traits.

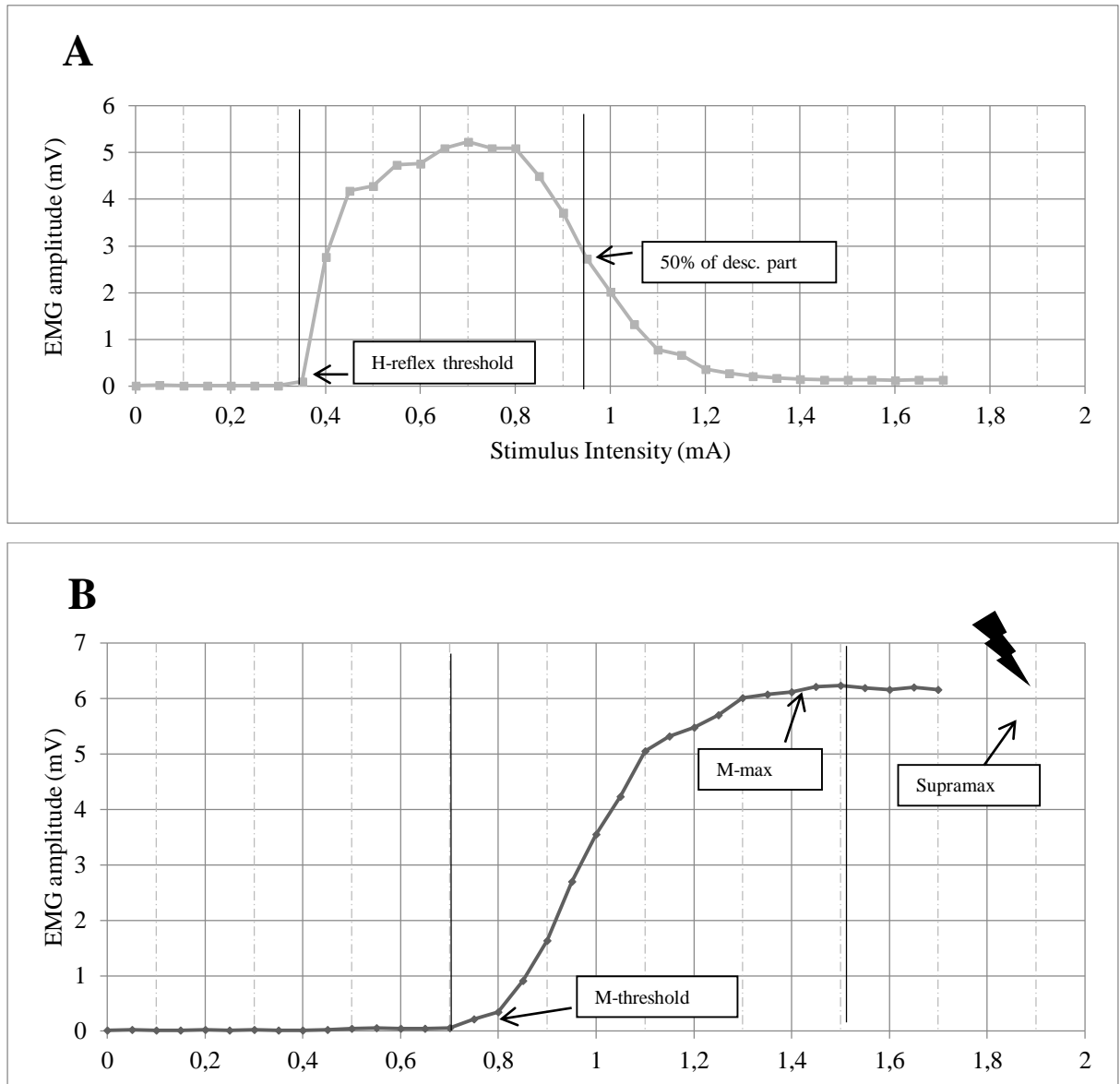


FIGURE 18. Example curves with marked threshold positions. A: H-reflex curve; B: M-wave curve.

The resulting recruitment curve contained enough stimuli to calculate the following parameters (Figure 20):

- maximal reflex amplitude - H_{\max}
- maximal muscle response - M_{\max}

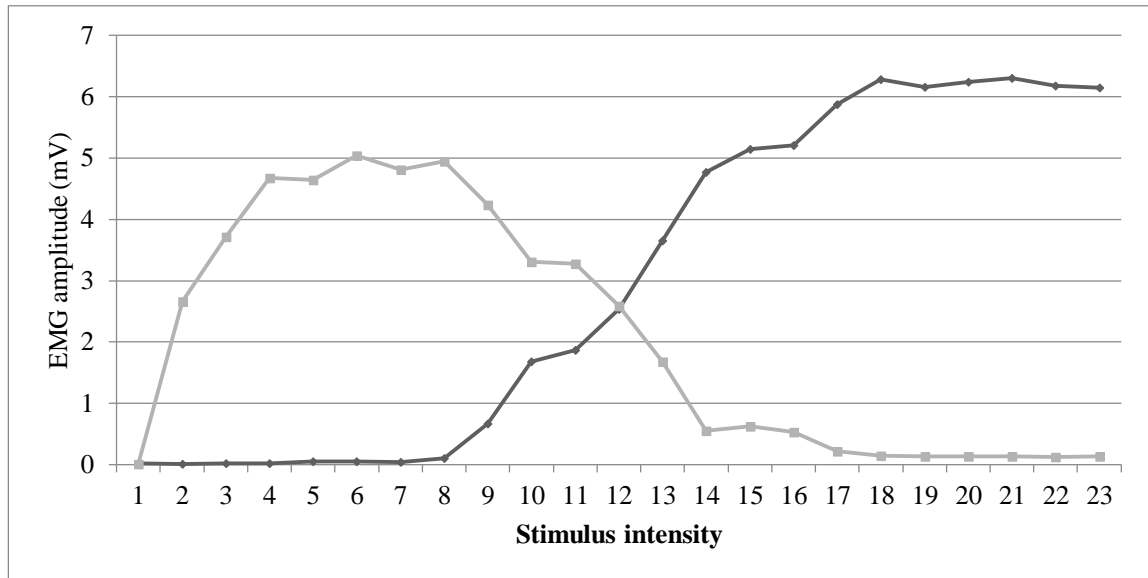


FIGURE 19. Fast recruitment curve contained enough information to record reflex excitability changes in different conditions.

For the fast recruitment curve, the order of stimuli was randomized to avoid the effect of anticipation (Ivanchenko 2014; Burke & Kamen 1995). In order to avoid post-activation depression, interstimulus interval was set between 8-12 seconds with custom made script (Zehr 2002). The order of the stimulation train was also changed for every condition. Thus, subjects were not able to learn or recognize the stimulation pattern in repeated measurements. Full fast recruitment curve was completed in a maximum of 4 minutes, with the primary aim of obtaining enough information in the least amount of time.

In order to record muscle architecture changes during the protocol, ultrasound images were recorded from the soleus muscle after each H-reflex recruitment curve. A personal computer-based portable ultrasound system (Echoblaster 128; Telemed, Vilnius, Lithuania) was used with a 96-element linear probe (B-mode; 6MHz, 50 mm transducer field width, sampling frequency 80 Hz), which was placed over the right leg to estimate fascicle length change of the soleus during dry land and water conditions (Figure 20). The probe was held over the same position by the same assistant for each recording. During dry land conditions, acoustic gel was used (Aquasonic gel 100, Parker Laboratories Inc., USA), while during water conditions, based on preliminary testing, no additional gel was required for the recording.

Ultrasound recording was not possible in every test session, so data were pooled into a single recording.



FIGURE 20. Position marking of the ultrasound probe during immersed condition.

Analysis

The recorded H-reflex and M-wave signals were analyzed trial by trial. Maximal H-reflex amplitudes were expressed in relation to the corresponding maximal M-wave peak-to-peak amplitudes (H/M ratio). Analysis was done using a custom written Matlab script (Mathworks).

Ultrasound images were analyzed using ImageJ software (Wayne Rasband, National Institutes of Health, USA). Muscle thickness value was the average of three recording site: distal, medial and proximal part of the image (Figure 21).

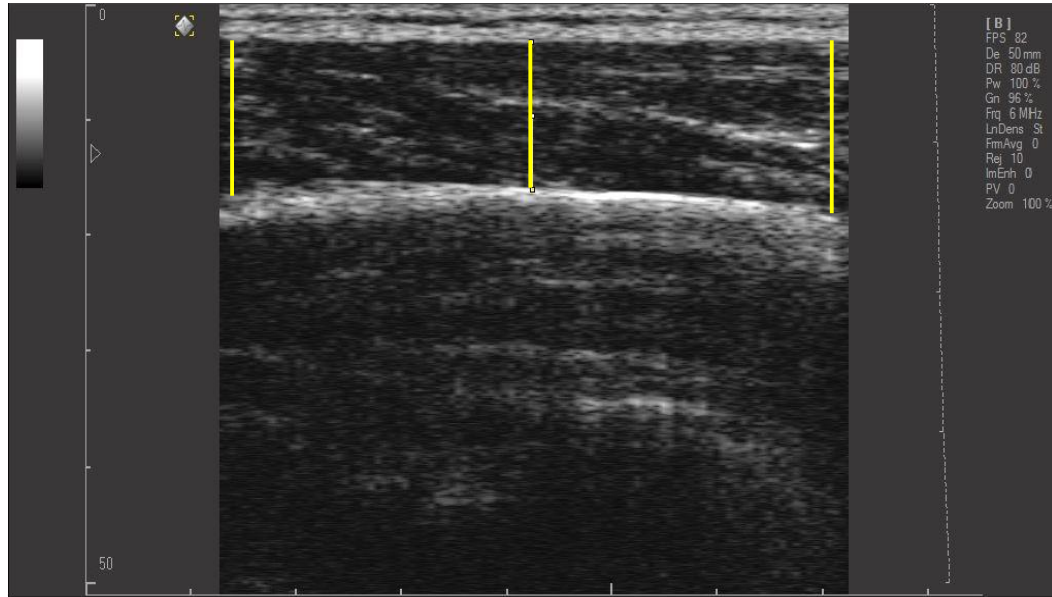


FIGURE 21. Illustration of an ultrasound image and the analysis procedure.

4.3 Statistical analyses

Standard procedures were used to calculate means, standard deviations (SD), and the coefficients of variation (CV). One-way repeated measures ANOVA was used to examine differences in maximal H/M ratio, maximal M-wave and fascicle length over the course of 1 hour on dry land and in water. Three-way repeated measures ANOVA was used to examine whether water immersion elicited changes in maximal H/M ratio, maximal M-wave, maximal H-reflex and muscle thickness over the one hour sessions on the two days.

Normality of the data distribution and presence of outliers were tested using the Shapiro-Wilk's W-test. Sphericity was assessed using Mauchly's test of sphericity. If the assumption of sphericity was violated, Greenhouse & Geisser correction was used. In cases where ANOVA main effects were significant, post-hoc tests (Bonferroni–Dunn) were performed. For all tests, the significance level was set at $p < 0.05$. Between day reliability (ICC [3.1]) was assessed for H/M ratio, maximal H-reflex and maximal M-wave. As ultrasound data were not recorded from every test session only within session reliability of fascicle length measures was calculated during the dry land recording. Confidence intervals of 95% were also calculated for ICCs.

5 RESULTS

Data of maximal H/M ratio, maximal M-wave, maximal H-reflex and thickness was normally distributed. There were no outliers in maximal H/M ratio; however, there were 4 outliers in the maximal M-wave data and one outlier in the fascicle length data. Inspection of their values did not reveal them to be extreme and they were kept in the analysis.

5.1 Within condition analysis

One-way repeated measures ANOVA showed no difference over time in maximal H/M ratio, maximal M-wave and H-reflex on Day0 or Day7 for during dry land recording. One-way repeated measures ANOVA showed no difference over time in maximal H/M ratio during water condition either on Day0 and Day7 (Figure 24D), but a one-way repeated measures ANOVA showed statistically significant difference over time in maximal M-wave amplitude during water immersion on Day0 ($F(1.292, 12.916) = 10.935, p \leq 0.001$, partial $\eta^2 = .0522$) and on Day7 ($F(1.75, 15.747) = 3.987, p = 0.009$, partial $\eta^2 = .0307$, Figure 24B). Post hoc analysis with a Bonferroni adjustment revealed that there was a significant reduction in maximal M-wave amplitude on Day0 during immersion between 0 and 60 minutes (mean difference of 0.624 mV (95% CI, 0.035 to 1.212), $p = 0.035$). One-way repeated measures ANOVA showed statistically significant difference over time in maximal H-reflex during water immersion on Day0 ($F(4, 40) = 12.033, p \leq 0.001$, partial $\eta^2 = .0546$) and on Day7 ($F(1.931, 17.383) = 3.758, p = 0.045$, partial $\eta^2 = .0295$, Figure 24C). Post hoc analysis with a Bonferroni adjustment revealed that there was a significant reduction in maximal H-reflex on Day0 during immersion between the 0 and 30, 45 and 60 minutes (0 vs. 30 minutes - mean difference of 0.537 mV (95% CI, 0.099 to 0.975), $p = 0.013$; 0 vs. 45 minutes - mean difference of 0.491 mV (95% CI, 0.063 to 0.919), $p = 0.021$; 0 vs. 60 minutes - mean difference of 0.551 mV (95% CI, 0.166 to 0.937), $p = 0.005$) and on D7 during immersion between the 0 and 45 minutes (mean difference of 0.380 mV (95% CI, 0.003 to 0.757) mV, $p = 0.048$).

One-way repeated ANOVA showed no difference over time of muscle thickness during dry land or water immersed recording (Figure 24A).

5.2 Between condition analysis

A three-way repeated measures ANOVA was run to determine the effect of water immersion on soleus maximal H/M ratio, maximal M-wave and maximal H-reflex peak-to-peak amplitude within Day0 and Day7. There was no difference in maximal H/M ratio between the two recording days, between the two environmental conditions and the elapsed time had no main effect on the variable (Figure 22 and 24D).

There was no difference in maximal M-wave amplitude between the two recording days (Figure 24B). Water immersion significantly decreased peak-to-peak amplitude of the maximal M-wave ($F(1, 9) = 10.948$, $p = 0.009$, partial $\eta^2 = .0.549$), with a mean difference of 0.816 V (95% CI, 0.258 to 1.374). There was no difference in maximal H-reflex between the two recording days and between the two environmental conditions (Figure 24C).

A two-way repeated measures ANOVA was run to determine the effect of water immersion on soleus muscle thickness. There was a statistically significant reduction in muscle thickness in water immersed condition ($F(1, 7) = 142.444$, $p \leq 0.001$, partial $\eta^2 = .0.953$, Figure 23 and Figure 24A) with a mean difference of 1.5 mm (95% CI, 1.2 to 1.8).

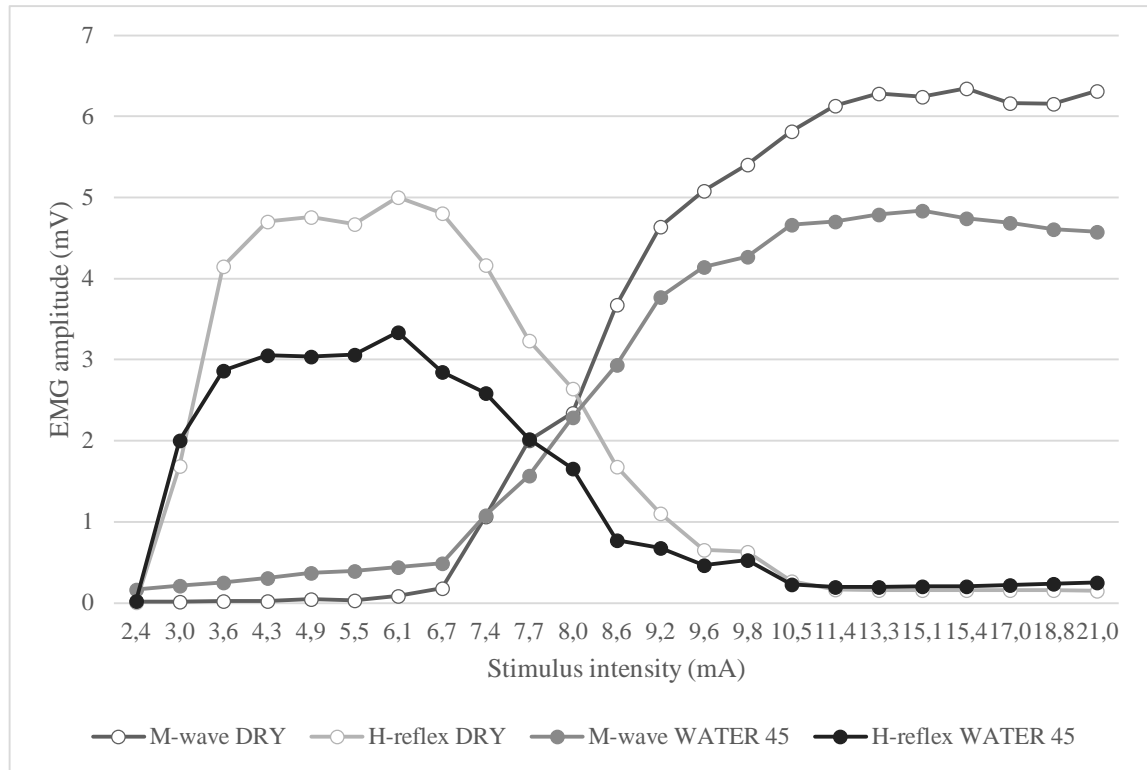


FIGURE 22. Example recruitment curve of Hoffmann reflex and M-wave between dry and water condition from 1 subject (45 minutes after immersion, n=1).

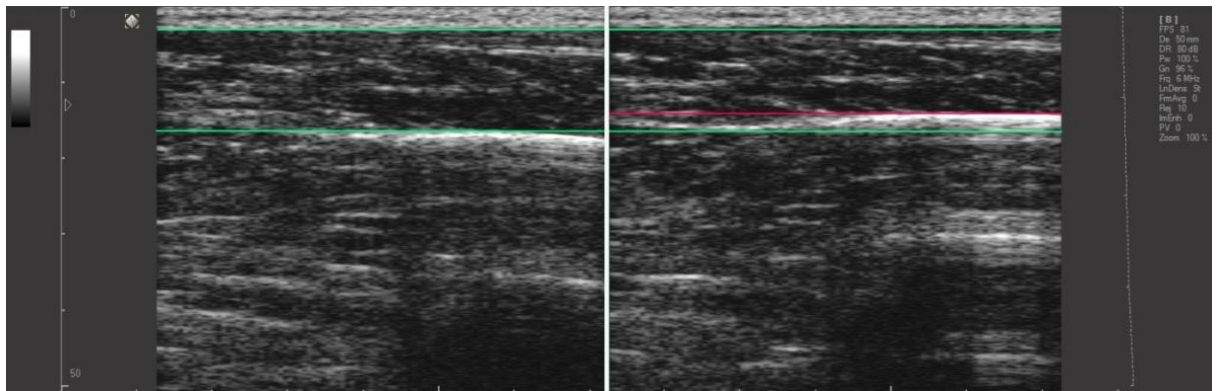


FIGURE 23. Example image of ultrasound recording. Left picture shows resting, dry land muscle, while the right picture was taken approximately after 5 minutes of immersion.

5.3 Between day difference and reliability

Two-way repeated measures ANOVA showed no significant difference between Day0 and Day7 in maximal H/M ratio in the two different environmental conditions.

Test-retest reliability (ICC) was calculated between days comparing the same recording time points for maximal H/M ratio, M-wave and H-reflex. The between-day reproducibility coefficients of maximal H/M recordings are shown in Table 2. Mean ICC for dry land maximal H/M ratio was 0.825 ± 0.052 and for water immersed condition it was 0.819 ± 0.104 . Two-way repeated measures ANOVA showed no significant difference between Day0 and Day7 in maximal M-wave amplitude in the two different environmental conditions. The day-to-day reproducibility coefficients of maximal M-wave are shown in Table 3. Mean ICC for dry land maximal M-wave was 0.714 ± 0.027 and for water immersed condition it was 0.512 ± 0.088 . Two-way repeated measures ANOVA showed no significant difference between Day0 and Day7 in maximal H-reflex amplitude in the two different environmental conditions. The day-to-day reproducibility coefficients of maximal H-reflex are shown in Table 4. Mean ICC for dry land maximal H-reflex was 0.860 ± 0.03 and for water immersed condition it was 0.668 ± 0.088 .

The intraclass correlation coefficient of muscle thickness on dry land was $ICC = 0.876$, $CI\ 95\% = 0.713-0.969$. As the data showed significant reduction of muscle thickness during immersion, intraclass correlation coefficient was not calculated in water condition.

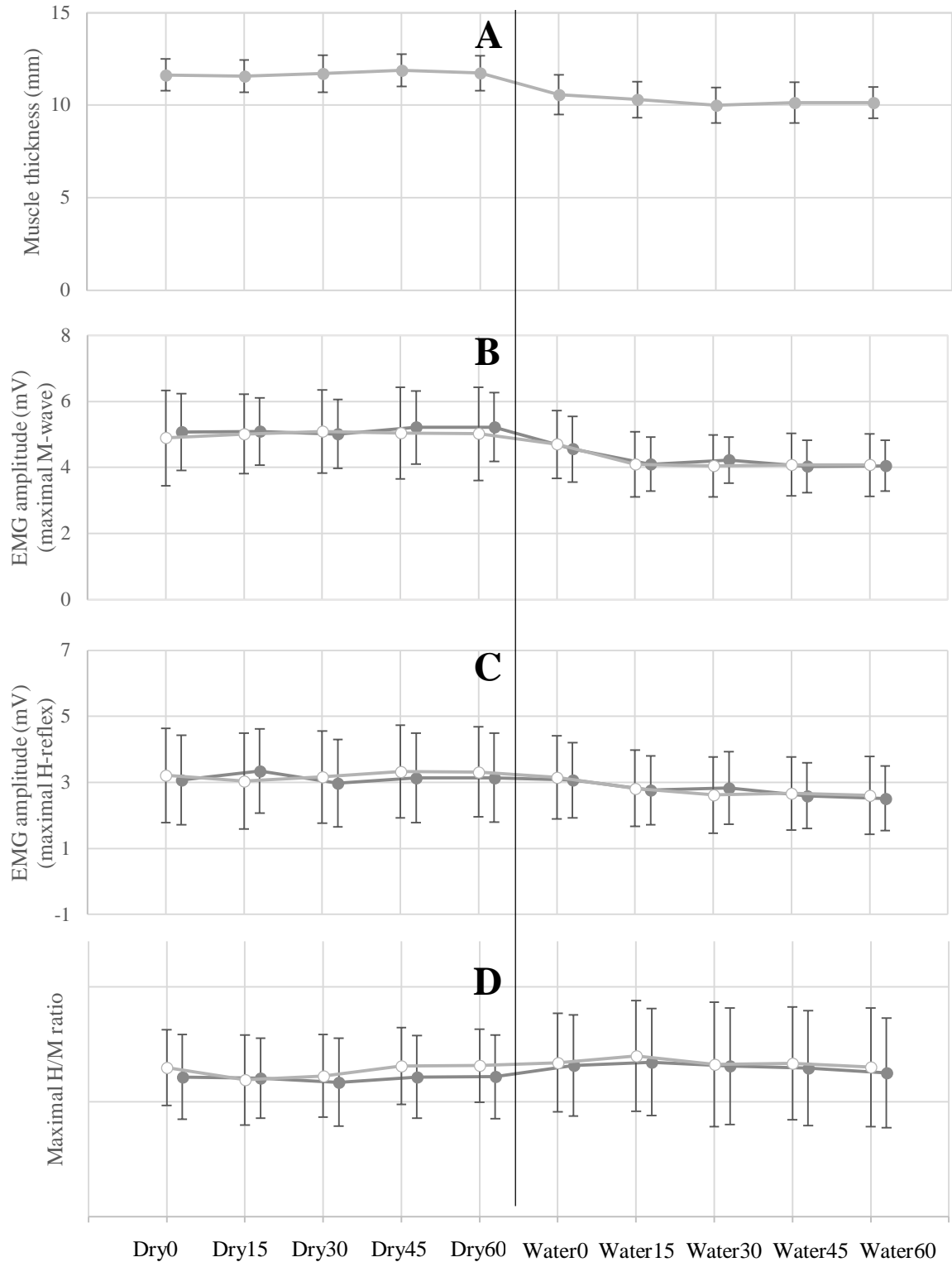


FIGURE 24. Mean changes of muscle thickness (A), maximal M-wave (B), maximal H-reflex (C) and maximal H/M ratio (D) over time (vertical line indicating the time of immersion). Non-filled circles indicate the recording from Day0, while grey, filled circles indicate Day7 recordings. Standard deviation of the whole population has been marked.

6 DISCUSSION

The main findings indicate that shallow, thermoneutral water immersion has no immediate effect on the maximal H-reflex amplitude, while the maximal M-wave and muscle thickness decreased significantly. Water immersion had no effect on maximal H/M ratio during 1-hour long period. Maximal M-wave and muscle thickness showed rapid decrease due to immersion and after 15 minutes there were no further decrease. Results show stable maximal H-reflex, maximal M-wave and muscle thickness on dry land. Pairwise comparison of the recording points within the two days shows good repeatability of the maximal H/M ratio. Maximal H-reflex' repeatability was good on dry land and moderate during immersion, while maximal M-wave' repeatability was moderate in both conditions. Muscle thickness showed good inter-session reliability on dry land.

The first hypothesis of thesis that maximal H/M ratio increases during immersion was refused, but the second hypothesis was accepted as muscle thickness decreased significantly due to immersion. The third hypothesis was accepted as the recorded parameters remained stable during dry land recording, and the fourth hypothesis also accepted as the repeatability shows moderate to good reliability over one week in maximal H/M ratio, maximal H-reflex and maximal M-wave amplitude.

6.1 Neuromuscular and muscular changes during thermoneutral water immersion

Normalizing the H-reflex to the maximal M-wave (H/M ratio) is a common method to assess neuromuscular changes and motoneuron pool excitability (Koceja et al. 1995; Zehr 2002; Knikou 2008). During one hour long thermoneutral water immersion the maximal H/M ratio didn't change significantly in young healthy male subjects. However, our data shows 8% mean increase in water compared to dry land condition. The increase of maximal H/M ratio tended to appear immediately in the first 5 minutes of immersion and peaked at 15 minutes with an average 11.5% increase compared to dry land. These results show similar pattern to

what Cronin et al. (2016) found in their study. Their findings show an immediate increase in maximal H/M ratio in healthy and hemiplegic subjects. After returning to dry land maximal H/M ratio decreased non-significantly after five minute, compared to control value (Cronin et al., 2016). In our study we didn't record post-immersion Hoffmann reflex.

Analyzing the two components of the maximal H/M equation shows that the two parameters behave differently during immersion (Figure 24D). At the first 5 minutes of immersion average maximal M-wave decreased non-significantly compared to control, dry land condition. On the other hand, no decrease can be seen on the maximal H-reflex. As the numerator remains the same and the denominator decreased, the H/M ratio increased non-significantly. After 15 minutes of immersion the maximal M-wave showed significantly lower peak-to-peak amplitude compared to dry land. Maximal H-reflex tended to decrease at the same time. Maximal H/M ratio showed the highest increase at 15 minutes of immersion. After 30 minutes of immersion the maximal M-wave showed steady state level, while maximal H-wave tended to decrease until 45 minutes of immersion (Figure 25B and C). As the denominator remained the same and the numerator decreased, the maximal H/M ratio decreased, reaching the level of dry land value. Immediate increase in maximal H/M ratio is in contrast to what Pöyhönen and Avela (2002) reported, that an approximately 30% reduction appears during immersion. However, the different protocol setup in that study such as deeper level of immersion, colder water (Krause et al. 2000) and compressive support (Goldberg et al. 1992) and taping could be the source of the different outcome.

Distortion of the EMG signals because of the effect of water leakage or noise caused by the immersion is less likely. During the recording EMG signals were monitored and no changes in signal-to-noise ratio were noticed. The fact that the decrease of maximal M-wave are continuous until a certain point, where after no more decrease were recorded also supports the idea of the non-disturbed EMG recording. Analysis of the muscle thickness showed rapid decrease during immersion (Figure 24A). After 5 minutes of immersion there was a significant reduction ($p=0.007$ with a mean difference of 1.2 mm (95% CI of 0.3 to 2) of muscle thickness. Decrease of muscle thickness last longed until 30 minutes of water

immersion (Figure 24A). Both the muscle thickness and maximal M-wave shows similar trend in decrease during water immersion (Figure 24A, 24B).

As it can be seen from the literature, maximal M-wave and muscle thickness remains stable in various joint angle positions if there is no voluntary contraction, while the H-reflex amplitude decreases in different joint positions. In the present study, during the dry land recording the H/M parameters and muscle thickness remained stable which suggests the reliability of the protocol.

Decrease of muscle thickness due to immersion could be the result of weightlessness or the water pressure. The latter is less likely as the level of immersion was around 20 cm which is equal to 15 mmHg along the muscle and previous study showed no vascular pressure increase at this level (Miki et al. 1989). Dry immersion and water immersion studies show decreased stiffness and muscle tone in skeletal muscles due to the effect of weightlessness (Navasiolava et al. 2011; Adams et al. 2003). However, full comparisons of these results are not possible because of the highly different protocol setups (whole body dry immersion, protocols usually last longer than 24 hours without recording of acute effects).

There is a general EMG activity decrease during immersion with both surface and indwelling electrodes (Cuesta-Vargas and Cano-Herrera 2014; Masumoto et al. 2004; Pöyhönen & Avela 2002), yet the nature of the changes is unknown. Our results suggest that the origin for these changes is muscle architecture. The changes in muscle architecture (muscle pennation angle and fiber length) could alter the action potential potentiation, which causes the decrease of M-wave by reducing the force capacity of the muscle. On the other hand, if the changes in muscle thickness and architecture lead to the overall decrease in EMG activity it would be expected that the H-reflex and M-wave decrease in a similar pattern. Results suggest that M-wave shows parallel decrease as the muscle thickness, but not the H-reflex. Therefore, it could be proposed that, in the present experiment the lack of reduction in H-reflex during water immersion is due to an increase of motoneuronal excitability or reduction of activity of inhibitory pathways. Further, according to the results these neural changes are the highest in the first 15 minutes of immersion. Iles (1996) recorded decreased presynaptic inhibition

while cutaneous stimulation was applied to the plantar surface of the foot in both cat and human subjects. Although this conclusion was more related to movement, the water could stimulate effectively these skin areas and remove the presynaptic inhibition and increase reflex amplitude. Water immersion increases activity in the primary somatosensory area after 20 seconds of water immersion and parietal association area after 40 seconds of immersion (Sato et al. 2012). Further descending depression of presynaptic inhibition could maintain the increased reflex response (Rudomin & Schmidt 1999). Effect of water immersion overall could activate cutaneo-muscular receptors (Meissner's and Pacinian corpuscles) which through inhibitory interneurons can suppress the primary afferent depolarization and decrease presynaptic inhibition. This way the flow of information could increase in the afferent pathways, activating the sensory cortex which through corticospinal pathway can enhance the suppression of presynaptic inhibition. The combined effect of the decrease from muscle architectural changes and the compensatory effect on reflex size due to decreased presynaptic inhibition may result in a compensated maximal H-reflex amplitude.

From the data it is not possible to determine the precise neurophysiological changes that appear during immersion, however the results suggest which parameters should be addressed in later studies. To ensure the effect of water stimulation without water pressure we suggest the use of a hose or flowing water on the surface of the skin. As for mimicking water pressure, an air cuff system can be used without immersion. These two protocols can help to distinguish which part of the water immersion elicits the stimulation. Basic experimental procedure to test presynaptic inhibition during water immersion could also be used (Iles 1996; Hultborn et al. 1987). Another possible mechanism underlying water immersion is the fluid shift caused by the water pressure. We did not record fluid shift changes but for future study plans it is recommended to use bioelectrical impedance analysis (Berg et al. 1993) to ensure if the possible background mechanisms are related to it.

6.2 Reproducibility of Hoffmann reflex, maximal M-wave and muscle thickness

The test-retest reliability (ICC) of the maximal H/M ratio was found to be similar on dry land and during water immersed condition, ranging from 0.66 to 0.94. Averaging the ICC values of the two environmental conditions, the results shows 'good' level of reliability in both conditions (>0.80) (Atkinson & Nevill 1998). Reproducibility of the maximal M-wave values shows 'moderate' reliability during water immersed condition with an average ICC of 0.51, and 'moderate' reliability on dry land (mean ICC of 0.71), ranging from 0.46 to 0.73. Maximal H-reflex amplitude shows 'good' reliability on dry land condition with a mean ICC (3.1) of 0.86, ranging from 0.81 to 0.91. However, in water immersed condition, maximal H-reflex has 'moderate' reliability with a mean ICC of 0.66.

Chen et al. (2010) reported similar reproducibility between 3-7 days in maximal H/M ratio (0.86) in neutral position, during rest, while the maximal M-wave and H-reflex showed 'excellent' reliability in their study. Ali and Sabbahi (2001) showed 'poor' (ICC 0.29) reliability of maximal H-reflex amplitude within 10 days period in prone position and high reliability of H-reflex latency in within day and between day. Compared to our results Christie et al. (2004) and Handcock et al. (2001) also recorded higher reliability of maximal M-wave response between sessions. Possible explanation for the lower reliability of maximal H-reflex and M-wave values that both are absolute values and highly depends on skin preparation, skin-electrode interface. It is possible that the location of the electrodes shifted over the one-week as the subjects were not able to keep the markers on the same position.

The test-retest reliability (ICC) of the soleus muscle thickness within the one-hour dry land testing was 0.88. Crofts et al. (2014) reported 0.90-0.97 intraclass correlation coefficient for foot muscles thickness, while Thoirs and English (2009) shows 0.83 ICC for posterior lower leg. In the latter study highly experienced sonographer reached similar reliability as in our study with less experience.

We have no knowledge about a study that would have tested the reliability of underwater Hoffmann reflex. Our results show similar reliability of maximal H/M ratio as previous studies on dry land and the results suggest similar repeatability in water as well. Compared to the literature, maximal M-wave recording showed ‘poor’ reliability in both environmental conditions. Possible explanations can be that the electrode position was not maintained and/or the skin-electrode interface was different between sessions. Permanent marker was used to mark the electrode positions for each subjects, however it could be possible that for the second session the position has been changed. This could lead to the alteration in the absolute values of maximal M-wave and H-reflex but not for the maximal H/M ratio. Another possible reason for the lower repeatability during immersion is that neuromuscular system has day-to-day difference in response for immersion.

As it has been presented in the literature review there are numerous factors that could alter the Hoffmann reflex, like training, age, protocol set-up. Our results show no alteration during dry land session which supports the idea of the non-altered condition and lead to suggestion that the changes that appear during immersion are due to the altered environmental conditions and not to errors in the protocol.

7 MAIN FINDINGS AND CONCLUSIONS

The main findings and conclusions of the present study can be summarized as follows:

- I. Maximal H/M ratio of the soleus muscle non-significantly increased acutely during 1- hour long thermoneutral water immersion. Maximal muscle response (M-wave) and muscle thickness decreased during the first 15 minutes of recording. Maximal H-reflex remained unchanged in the first 5 minutes of water immersion and showed small decrement during one-hour long immersion. Possibly the effect of weightlessness and/or the cutaneous stimulation of water and water pressure caused the decrease of presynaptic inhibition and counteract/compensated the H-reflex decrease due to the muscle architecture changes. Maximal H/M ratio returned to control level at the end of the one-hour long protocol.
- II. Ultrasonography recording of the soleus muscle thickness showed acute changes during water immersion. In the first 15 minutes of immersion the muscle thickness decreased significantly until it reached a steady state level. Decrease of muscle thickness is caused by the relative weightlessness and/or water pressure.
- III. During dry land testing all the recorded parameters (maximal H/M ratio, maximal M-wave, maximal H-reflex, muscle thickness) remained unchanged. The results indicate that in future studies one control recording is enough on dry land.
- IV. Day-to-day repeatability of the maximal H/M ratio showed similar results as previous studies have reported. On both dry land and water immersed condition the reproducibility was acceptable. Maximal M-wave on dry land and in water immersed condition showed poor repeatability, while the maximal H-reflex responses on the two environmental conditions are acceptable. Muscle thickness within condition reliability is acceptable.

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APPENDIX

TABLE 1. Review table of the test-retest reliability of the Hoffmann reflex: the following information has been extracted: which muscle has been tested; in what position do they tested the reflex; time between tests; size of the subjects group and their average age; properties of the electrical stimulus and its increment; variables that has been measure and calculated; reliability coefficients. Studies marked with * were only available in abstract form.

Reference	Muscle	Objective/Protocol	Subjects (mean age \pm SD; range)	Stimulus	Variable	Reliability of the measurement / Main outcome
Ali & Sabbahi, 2001*	Soleus	Test-retest and within-subject reliability of the soleus H-reflex in prone/lying, free standing and standing while lifting 20% bodyweight within 10 days.	15 participants (20-50 years old)	0-5ms pulse; 0.2 pps (=5s ISI)	Peak-to-peak amplitude and onset latencies of four separate traces	Test-retest reliability of H-reflex amplitude: prone position: 0.29; loading position: 0.56. Within day reliability of the H-amplitude between the three different positions: 0.56-0.97. Test-retest reliability of the H-latency: 0.92-0.94. Within day reliability of the H-latency: 0.96-0.99

Alrowayeh & Sabbahi, 2006	Vastus medialis (VM)	Intrasession and intersession reliability test of VM H-reflex amplitude while standing during 0, 30, 45 and 60-degree knee flexion for two constitutive days.	5 healthy subjects with no history of orthopedic disease	0.5ms; square wave; 0.2 pps (=5s ISI)	Peak-to-peak amplitude of H-reflex; ICC (2.4); ICC (2.1)	Intrasession reliability: 0 degree-day 1: 0.96-0.98; day 2: 0.94-0.98; 30, 45, 60 degrees-days 1: 0.90-0.97; day 2: 0.76-0.96. Intersession reliability: 0 degree: 0.77-0.84; 30 degree: 0.70-0.77; 45 degree: 0.51-0.75; 60 degree: 0.55-0.73
Alrowayeh & Sabbahi, 2009*	Gastrocnemius medialis (GM) and gastrocnemius lateralis (GL)	Intersession reliability of the GM and GL muscles during standing and lying postures at neutral, full dorsiflexion and full plantarflexion over two constitutive days.	8 healthy subjects (36 ± 6; 29-42 years)	1ms; 0.2 PPS (=5s ISI)	Hmax; Mmax	Intersession ICC (2.1) during standing and lying with varied ankle positions: 0.58-0.94. ICC of GM during standing: 0.76-0.94; during lying: 0.58-0.85.

Chen et al., 2010	Soleus	Test-retest reliability of soleus H-reflex during different voluntary force production levels and in different ankle joint angles in sitting position.	10 healthy subject (24.9 ± 5)	10mA increment (1-2mA at H_{max}); 10/30s ISI	H_{max} ; M_{max} ; H_{max}/M_{max} ratio	Intraclass correlation coefficient (2.1) at rest: H_{max} : 0° - 0.96; 20°- 0.98; -20°- 0.97; M_{max} : 0° - 0.96; 20°- 0.75; -20°- 0.89; H_{max}/M_{max} : 0° - 0.86; 20°- 0.96; -20°- 0.84. Intraclass correlation coefficients (2.1) during voluntary contraction: 0°- 10% MVC: 0.92; 0°- 30% MVC: 0.64; 0°- 50% MVC: 0.97; 20°-10% MVC: 0.93; 20°- 30% MVC: 0.66; 20°-50% MVC: 0.83; -20°- 10% MVC: 0.95; -20°- 30% MVC: 0.62; -20°- 50% MVC: 0.79
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Christie, Lester, LaPierre, & Gabriel, 2004	Soleus	Intraclass reliability of different measures extracted from Hoffmann reflex, measured in 5 days.	24 subjects (12 female and 12 male)	1ms; square wave; 4 V increment; 15s ISI; 5 stimulus/intensity	Mean P-P amplitude of 5 trials at each intensity; P-P amplitude of H response at 5% M_{max} ; H-slope (regression curve), ICC (2.k)	Intraclass correlation coefficient of: H-slope: 0.79; H5%: 0.85; M_{slope} : 0.94; M_{max} : 0.96;
Doguet & Jubeau, 2014*	Quadriceps vastus medialis, vastus lateralis	Evaluate the modulation and reliability of the selected muscles in active (30% MVC) and passive conditions in 3 sessions in 1 week.	12 healthy participants		Recruitment curves of VM, VL; H/M ratio; CV (absolute reliability); ICC (relative reliability)	Potentiated H-reflex in active condition; VM amplitude +150% compared to VL; Intra and inter-day reliability: ICC_{hour} : 0.97; ICC_{day} : 0.92; ICC_{week} : 0.92. Intra and inter-day reliability for the passive VM H/M ratio: CV_{hour} : 52.2%; CV_{day} : 69.8%; CV_{week} : 60.9%. Reliability in active condition: ICC_{hour} : 0.93; CV_{hour} : 12%; ICC_{day} : 0.86; CV_{day} : 14.5%; ICC_{week} : 0.79; CV_{week} : 19.7%

Handcock, Williams, & Sullivan, 2001*	Soleus	Reliability test of individual differences in the H-reflex during quiet standing and during prolonged testing. 8 blocks of 20 trials, distributed over two testing sessions.	20 healthy participant	10s ISI; 2 min seated rest between blocks	Peak-to-peak amplitude of H-reflex and M response	Reliability if measuring the H-reflex and M-response during quiet standing: 0.97. Reliability when 4 trials analyzed: 0.96 and 0.87.
Hayes, Hicks-Little, Harter, Widrick, & Hoffman, 2009	Soleus	Determination of day-to-day reliability of the soleus H-reflex gain and presynaptic inhibition of spinal reflexes between two testing sessions in prone position.	15 healthy male (23.7 ± 4.3) and 15 healthy female (22.8 ± 3.7)	1ms	H-reflex gain: ratio of the slope of the ascending portion of the H-reflex and M-wave (H_{slp}/M_{slp}); Presynaptic inhibition; ICC(2.1)	ICC of the H_{slp}/M_{slp} : 0.951; ICC of the presynaptic inhibition: 0.912

Hopkins & Wagie, 2003*	Quadriceps	Reliability of the quadriceps muscle over a four-week period consisting of 6 testing sessions (First session, 1 hour, 24 hour, 1, 2, 3 weeks following).	11 neurologically sound volunteers (20 ± 2)	20s ISI	Peak quadriceps H-reflex normalized to maximal M-response; ICC (3.1) and ICC (2.1)	Within session reliability: 10 trials ICC (2.1) = 0.957; ICC (3.1) = 0.970; 5 trials ICC (2.1) = 0.961; (ICC (3.1) = 0.970. ICC (2.1) reliability within session: day 1 first and second (1hr) session: 0.956; day 1 and 2: 0.787; between all session over 4 weeks: 0.756. ICC (3.1) reliability for the fixed selection of sessions: between day 1 and 2: 0.969; between all sessions over 4 weeks: 0.911
Mynark 2005	Soleus	Inter-trial reliability of the soleus H-reflex in young and elderly group during supine and standing position.	10 young healthy (23,2 ± 2.5 years); 10 healthy elderlies (69.1 ± 5.2 years)	1 ms; square wave; 15-30s ISI	Relative H-reflex (standardized to 25% Mmax); H-max; M-max;H/M ratio	Intraclass Correlation (2.1) of relative H-reflex of 10 trials: Y-Sup: 0.99; Y-Sta: 0.97; E-Sup: 0.97; E-Sta: 0.91

Phadke et al. 2010*		Reliability of soleus H-reflex in individuals with incomplete spinal injury (SCI) during the standing and the swing and stance phases of over ground walking (self-selected speed) tested on two separate days.	14 SCI subject (40 ± 10 years); 8 non-injured subjects (32 ± 9)		H-reflex; Intraclass correlation (1,2); Standard error of measurement (SEM)	ICC of the H-reflex in non-injured and SCI subjects: 0.64-0.91; SEM expressed as percentage of the mean H-reflex: non-injured: 13%-62%; SCI: 12%-18%
Raglin, Koceja, Stager, & Harms, 1996	Soleus	Effect of seasonal changes in training load on mood, neuromuscular function and physical power during competitive swim season (5.5 month).	12 collegiate female swimmers (19.3 ± 1.3)		H-max, M-max and H/M ratio	Day-to-day test-retest reliability intraclass coefficients: 0.92-0.97
Simonsen and Dyhre-Poulsen 2011*	Soleus	Test-retest ability of the soleus H-reflex during walking (4.5 km/h) separated by days.	7 male subject (30; 26-34)	1ms; square pulse; 2ms ISI during walking	H peak-to-peak amplitude normalized to Mmax; Spearman's Rho	Normalized H-reflex amplitude was significantly lower on Day 2; Spearman's Rho between the two days, normalized to Mmax: 0.92; during running: 0.88

Stowe et al. 2008	Flexor carpi radialis (FCR); Extensor carpi radialis longi (ECRL)	Reliability test in between 3 consecutive days in seated position.	8 healthy subjects (36 ± 6; 29-42 years)	1 ms; 5s ISI; 0,5mA increment; 3 pulse/intensity; holding a 0,5 lb weight	HTH - H-reflex threshold; HGN - H-reflex gain; HPP - peak-to-peak amplitude; visHTH - visual detection technique; sdHTH - calculated in Matlab	Interclass correlation coefficient of ECRL: Hmax: 0,94; HGN: 0,68; HTH: 0,97; visHTH: 1,00; sdHTH: 1,00; Interclass correlation coefficient of FCR: Hmax: 0,99; HGN: 0,57; HTH: 0,69; visHTH: 0,94; sdHTH: 0,99
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TABLE 2. Day to day reproducibility of absolute maximal H/M ratio between the two measurement days (ICC = intraclass correlation coefficient, CI 95% = 95-per-cent confidence interval).

	ICC [3.1]	CI 95%
Dry – 0 minutes	0.844	0.534-0.955
Dry – 15 minutes	0.744	0.324-0.923
Dry – 30 minutes	0.803	0.425-0.943
Dry – 45 minutes	0.866	0.479-0.965
Dry – 60 minutes	0.869	0.451-0.966
Water – 0 minutes	0.789	0.389-0.939
Water – 15 minutes	0.874	0.599-0.964
Water – 30 minutes	0.662	0.116-0.897
Water – 45 minutes	0.940	0.777-0.985
Water – 60 minutes	0.833	0.455-0.956

TABLE 3. Day to day reproducibility of absolute maximal M-waves between the two measurement days (ICC = intraclass correlation coefficient, CI 95% = 95-per-cent confidence interval).

	ICC [3.1]	CI 95%
Dry – 0 minutes	0.723	0.252-0.917
Dry – 15 minutes	0.721	0.232-0.917
Dry – 30 minutes	0.736	0.263-0.922
Dry – 45 minutes	0.727	0.259-0.919
Dry – 60 minutes	0.667	0.145-0.898
Water – 0 minutes	0.670	0.146-0.899
Water – 15 minutes	0.486	-0.174-0.833
Water – 30 minutes	0.464	-0.171-0.822
Water – 45 minutes	0.461	-0.257-0.837
Water – 60 minutes	0.483	-0.224-0.845

TABLE4. Day to day reproducibility of absolute maximal H-reflex between the two measurement days (ICC = intraclass correlation coefficient, CI 95% = 95-per-cent confidence interval).

	ICC [3.1]	CI 95%
Dry – 0 minutes	0.911	0.714-0.975
Dry – 15 minutes	0.843	0.539-0.955
Dry – 30 minutes	0.862	0.582-0.961
Dry – 45 minutes	0.818	0.469-0.947
Dry – 60 minutes	0.867	0.593-0.962
Water – 0 minutes	0.674	0.140-0.902
Water – 15 minutes	0.645	0.083-0.892
Water – 30 minutes	0.532	-0.076-0.849
Water – 45 minutes	0.754	0.256-0.933
Water – 60 minutes	0.738	0.221-0.929