CHARLES UNIVERSITY IN PRAGUE
Faculty of Physical Education and Sport

Physiological and Neuromuscular changes between young soccer players and untrained adolescents.
A comparison study

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Doctoral dissertation that is submitted to the professional body for the partial discharge of the obligations for the acquisition of the Doctoral Degree in Exercise Physiology of the Faculty of Physical Education and Sport of the Charles University in Prague.

Prague 2019
Candidate Declaration

This Doctoral dissertation constitutes my own work and all material that is not my own is fully acknowledged. No part of this work has been submitted for assessment elsewhere.

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Signed: Date: 20th July 2019

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Abstract

Athanasios Mandroukas: Physiological and Neuromuscular changes between young soccer players and untrained adolescents. A comparison study

Aim. The purpose of this study was to examine the effects of soccer training on maximal oxygen uptake, isokinetic muscle strength and anthropometric characteristics in different ages of soccer players and untrained adolescents of the same biological age.

Subjects. A total of one hundred and twenty six (n=126) young soccer players and untrained boys throughout the developmental ages of 12 (soccer players n=22; untrained boys= 22) 14 (soccer players n=20; untrained boys= 18) and 16 (soccer players n=22; untrained boys= 22) volunteered to participate in the study. Sexual maturation was classified according to Tanner’s stages. Soccer players participated both in their school’s physical education program and in a soccer training program, while the untrained participated only in their school’s physical education program.

Methods. All participants underwent anthropometric measurements and performed a maximal exercise testing on a motor driven treadmill to determine maximal oxygen uptake ($\dot{V}O_2_{\text{max}}$) and cardiorespiratory indices. Blood lactate concentration was determined in the 5th minute of recovery using a lactate photometer. The isokinetic concentric peak torque values of the hamstrings (H) and quadriceps (Q), as well as the conventional strength ratios of H:Q, were measured on an isokinetic dynamometer (CSMI, Humac Norm, Cybex II) at angular velocities of 60,180, and $300^\circ \cdot \text{s}^{-1}$.

Results. The trained group showed significantly higher $\dot{V}O_2_{\text{max}}$, in absolute and relative values (p<0.001), $\text{BL}_{\text{max}}$ (p<0.05) and $\text{RER}_{\text{max}}$ (p<0.05) compared to the untrained group. Resting HR and systolic blood pressure were significantly lower (p<0.05) for the trained compared to untrained. The isokinetic muscle strength (absolute and relative) was significantly higher (p<0.001) in the 12 and 16 years old trained group, compared to untrained, for the knee extensors and knee flexors. However, no significant differences were found between the 14 years
old trained and untrained, for the muscle groups of Q and H. The H:Q strength ratios did not differ between groups at all angular velocities.

**Conclusion.** The results of this study showed that systematic soccer training (intensity and duration) has a positive effect in the central cardiovascular system expressed as $\dot{V}O_2\text{max}$, HR and blood pressure, as well as in the peripheral system, expressed as an increase lower limb muscle strength; specifically, agonist - antagonist (Q and H). The results provide important information and knowledge for more effective training programs not only for soccer but also for any other sport training.

**Key words:** isokinetic muscle strength; hamstrings; quadriceps; H:Q strength ratio; maximal oxygen uptake; biological age; youth soccer players; exercise testing; developmental ages, untrained adolescents
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<thead>
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<tr>
<td>*</td>
<td>U12 trained vs U12 untrained, p&lt;0.05</td>
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<tr>
<td>**</td>
<td>U12 trained vs U12 untrained, p&lt;0.01</td>
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<tr>
<td>***</td>
<td>U12 trained vs U12 untrained, p&lt;0.001</td>
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<td>#</td>
<td>U14 trained vs U14 untrained, p&lt;0.05</td>
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<td>###</td>
<td>U14 trained vs U14 untrained, p&lt;0.001</td>
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<td>$</td>
<td>U16 trained vs U16 untrained, p&lt;0.05</td>
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<td>$$</td>
<td>U16 trained vs U16 untrained, p&lt;0.01</td>
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<td>$$$</td>
<td>U16 trained vs U16 untrained, p&lt;0.001</td>
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<td>†</td>
<td>U12 untrained vs U14 untrained, p&lt;0.05</td>
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<td>U12 untrained vs U14 untrained, p&lt;0.01</td>
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<td>§§§</td>
<td>U14 trained vs U16 trained, p&lt;0.001</td>
</tr>
<tr>
<td>NS</td>
<td>No significant differences</td>
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The symbols represent statistical significance levels, with * indicating p<0.05, ** indicating p<0.01, *** indicating p<0.001, and so on. The symbols for the untrained groups (†, ††, †††) follow the same pattern as for the trained groups.
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<th>Description</th>
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<tbody>
<tr>
<td>BCM</td>
<td>Body Cell Mass</td>
</tr>
<tr>
<td>BW</td>
<td>Body Weight</td>
</tr>
<tr>
<td>BF</td>
<td>Body Fat</td>
</tr>
<tr>
<td>PHV</td>
<td>Peak Height Velocity</td>
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<tr>
<td>H/Q ratio</td>
<td>Hamstrings to Quadriceps ratio</td>
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<tr>
<td>CA</td>
<td>Chronological age</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross-sectional area</td>
</tr>
<tr>
<td>H</td>
<td>Hamstrings</td>
</tr>
<tr>
<td>Q</td>
<td>Quadriceps</td>
</tr>
<tr>
<td>ST</td>
<td>Slow twitch muscle fibers</td>
</tr>
<tr>
<td>FT</td>
<td>Fast twitch muscle fibers</td>
</tr>
<tr>
<td>ROM</td>
<td>Range of Motion</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>CP</td>
<td>Creatine triphosphate</td>
</tr>
<tr>
<td>TC</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>(\dot{V}O_2)\text{max}</td>
<td>Maximal oxygen uptake</td>
</tr>
<tr>
<td>BLa\text{max}</td>
<td>Maximal blood lactate</td>
</tr>
<tr>
<td>Pcr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>LBM</td>
<td>Lean body mass</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>PT</td>
<td>Peak Torque</td>
</tr>
<tr>
<td>APT</td>
<td>Absolute peak torque</td>
</tr>
<tr>
<td>RPT</td>
<td>Relative peak torque</td>
</tr>
<tr>
<td>RER</td>
<td>Respiratory exchange ratio</td>
</tr>
<tr>
<td>(VCO_2 / \dot{V}O_2)</td>
<td>Respiratory exchange ratio</td>
</tr>
<tr>
<td>(\dot{V}O_2)</td>
<td>Oxygen uptake</td>
</tr>
<tr>
<td>(\dot{V}O_2)\text{(ml·min}^{-1}\text{)}</td>
<td>Absolute aerobic power</td>
</tr>
<tr>
<td>(\dot{V}O_2)\text{(ml·kg}^{-1}·\text{min}^{-1}\text{)}</td>
<td>Relative aerobic power</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>bpm</td>
<td>Beats per minute</td>
</tr>
<tr>
<td>D\text{BP}</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>S\text{BP}</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>RQ</td>
<td>Respiratory quotient</td>
</tr>
<tr>
<td>RPE</td>
<td>Rate of perceived exertion</td>
</tr>
<tr>
<td>SV</td>
<td>Stroke volume</td>
</tr>
</tbody>
</table>
List of Original Publications

The following original publication is based on this thesis and is attached in the appendix chapter/section:

Acknowledgements

I would like to take this opportunity to thank all those individuals who have made the completion of my Ph.D. thesis possible.

➢ I would like to thank all those amazing little guys, who voluntarily took part in the study. Without their time and commitment this would not have been possible.

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Athanasios Mandroukas
1. Introduction

During the last decades, a great emphasis is given to the role and significant profits of physical activity for children’s health (ACSM, 2006; Blair et al., 1989; Cavill et al., 2001; Dietz, 2004; Lee, 2003; Matthews et al., 2002; Sallis and McKenzie, 1991). Physical activity constitutes one of the most important factors for the normal growth of children and adolescents, as it influences positively the physical and psychological health and it is also very important for all the stages of the level in health and life quality. Researches have shown that children and adolescents, who participate in a certain physical activity have spiritual, psychological and bodily health (Hagger et al., 2001a, b), and that physical activity contributes to the maintenance of a physically active lifestyle (Krustrup et al., 2010). Regular soccer training, two to three times a week, caused significant cardiovascular and muscular adaptation (Nadeau et al., 2011; Krustrup et al., 2010). Fitness is also a strong indicator of health and it can also be considered as a major factor, affecting the structure and the efficiency of bodily exercise (Castillo et al., 2005; Ruiz et al., 2006).

The module of Physical Education in school is related to vigor and well-being, and it can constitute an important factor for the promotion of health in a modern society lifestyle (Sallis and McKenzie, 1991). Nevertheless, despite the recognized importance of exercise, the results of researchers of the last 25 years, show that the current way of life does not include sufficient physical activity and also that the children’s level of physical activity has been decreased (Armstrong, 1997; Armstrong et al., 1990, 2000; Dietz and Gortmaker, 1993; Sallis et al., 2000).
Worldwide, it has been observed, that the children’s and adolescent’s efficiency, in tests of muscular strength and velocity, has been decreased since 1990. There is also a characteristic decrease in the cardiovascular endurance in the various field test, in the developed countries (Tomkinson et al., 2003a, b). Although physical activity contributes positively in enhancing physical and psychological health of individuals of all years (U.S. Department of health and Human Services, 1996), it is true that a great percentage of children and adolescents are insufficiently active and that’s the reason they do not derive the desirable benefits for their health (Armstrong and Welsman, 2007; De Ste Croix et al., 2001). In a longitudinal study, when children’s physical activity was recorded in Finland (aged 12, 14, 16 and 18), it was found that the percentage of the very physically active children was reduced with the increase of age from 12 to 18 years: for the boys from 26% to 12% and for the girls from 13% to 5%. A lot of researchers report that the positive adaptations of exercise are greater during the phase of rapid growth (Shephard, 1982; Malina et al., 2004a). During the bodily growth and maturation, as well as during the growth of dexterities and behaviors, there are some periods, when children and adolescents are more sensitive to the stimuli from their environment, either positive or negative. Research has shown that the growth process can be more easily modified during these periods (Eccles et al., 1993).

Today, children start serious athletic training at younger age than ever before. Both in children and adults, soccer is one of the most popular team sports in the world (Wong and Hong, 2005). The aerobic capacity and the muscle strength in prepubertal and pubertal children have been a matter of long lasting controversy (Vamvakoudis et al., 2007). Some studies demonstrated an improvement in $\dot{V}O_2$ in both categories (Eriksson and Koch, 1973; Baxter – Jones et al., 1993), while other investigations (Bar – Or, 1983; Mirwald and Bailey, 1984; Williams and
Reilly, 2000) have shown little or no improvement. During puberty (13 – 16 years of age) differences in body size, aerobic power, muscle strength and performance occur between boys of contrasting maturity status, i.e. early versus late maturity of the same age (Malina et al., 2004b). Complicating factors in this controversy have been mainly the different experimental designs of the studies in this area as well as the chronological ages (CA) of the subjects (Armstrong et al., 1990). Most of these studies have been cross-sectional and therefore the effects of growth, development and heredity may have been greater than those of training (Vamvakoudis et al., 2001).

Training during childhood (prepubertal – pubertal) continues to be a subject of interest. However, reviewing the results of training with children of different CA, it is difficult to separate the effects of training from those of growth. Although the isokinetic muscle strength as well as the aerobic power have been studied extensively, little is the information on these variables throughout the same biological age in trained and untrained boys, who undertook only the physical activities in school. More specifically, little is known about the alteration that may occur in $\dot{V}O_2_{max}$ and muscle strength in trained and untrained groups. To the best of our knowledge, little is known about the effects of soccer training on aerobic power and concentric isokinetic strength of knee extensors and flexors in trained and untrained (non-athletic) adolescents of the same biological age. Many studies of young soccer players have not considered maturity status as a factor, influencing functional capacity (Chin et al., 1994). Some studies compared young soccer players with athletes of other sports, of different CA (Hansen et al., 1999), and others focused their investigation between young soccer players and adults (Rosch et al., 2000).

A research by Rochcongar et al. (1988) found that when it comes to muscle strength, the elite soccer players demonstrated a greater leg strength than the high-school students. The study
confirmed that soccer training affected the development of muscle strength positively. On the other hand, Maffulli et al. (1994) confirmed that athletic boys including soccer players below 15 years demonstrated the same isometric quadriceps’ strength similar to non-athletic boys. However, above 15 years, the strength of the athletic group increased as opposed to the second group. Soccer emerges as the most popular game in the world, and equally a valued sport by both the players and the spectators (Ekstrand et al., 2011; Bangsbo, 1994a). However, the game demands a specific type of physical activity classified as high-intensity intermittent exercise. Resultantly, the athlete’s body has to undergo both anaerobic and aerobic processes for the purpose of energy release. According to Åstrand et al. (2003), maximal oxygen uptake ($\dot{V}O_{2\text{max}}$) which is the maximum rate at which an individual can expend oxygen during exercise, reduces the ability to perform aerobic exercise and is known as the best single index of aerobic fitness. Maximal aerobic work capacity increases in elite soccer players. For this purpose, the relative supply of aerobic process accounts for 70-80% maximal capacity (Ekblom, 1986).

Soccer is a sport that demands power and strength, and therefore, the players need to gain strength through routine exercise. Such is essential to accumulate strength in most of the large muscle groups on the body. The strength is essential for various match activities such as kicking, jumping, tackling, turning, changing pace, and sprinting that is considered to be both forceful and explosive. The quadricep (Q) muscles of the knee joint play an important role in jumping and kicking, while the hamstring (H) muscles control the running activities and stabilize the knee turns (Fried and Lloyd, 1992). Some authors have suggested that H to Q strength ratios (H:Q) are good measures of normal knee function (Campbell and Glenn, 1982).

The present study was designed to examine and compare: a) the anthropometric characteristics; b) the maximal aerobic power; c) the absolute and relative muscle strength of the player.
knee extensors and knee flexors, as well as the H:Q strength ratio between young soccer players and untrained adolescents of the same biological age.

2. Literature Review

2.1 Biological Development

2.1.1 Maturity

Shakhanova et al. (2015), explain that soccer requires athletes to gain motor and cognitive skills and to integrate them in order to enhance performance. The mentioned prerequisites for effective soccer training emanate from biological development of body systems. Sexual development is an essential biological aspect for young adolescents, which influences numerous changes in the body. Body changes that arise from sexual development influence the process of soccer training among female and male adolescents between the ages of 10 and 15 years. Malina et al. (2015) explain that sexual development and skeletal age are essential indicators of biological maturation among young adolescents, indulging in sports training. They identify growth in height and muscle mass as an aspect of biological development among young athletes. According to the researchers, peak height in adolescents occurs due to such biological factors as increased production of growth hormone (GH) that results in an accelerated rate of muscle and overall body growth. Growth spurt among the young adolescents varies, but it mostly occurs between the ages of 9 and 15 years for both males and females. The rapid growth spurt in weight and height influences soccer training, because it elicits a positive impact on training intensity and endurance. Trainers should those recognise adolescent participants who have
attained the peak height velocity (PHV) and utilise it to improve soccer performance and enhance training.

Neuromuscular development is also identified as a vital biological development that influences soccer training among young adolescent between the ages of 11 and 13 years. Development in the neural and muscular system involves enhanced coordination of the brain (nervous system) and the muscles (muscular system). Neuromuscular development precedes skeletal growth and PHV as it primarily focuses on utilisation of both, for enhanced physical performance through the development of excellent motor skills. Trainers should acknowledge the variation in neuromuscular coordination in the developmental groups of 12 to 16 years and present an appropriate training program to facilitate efficient soccer performance (Hopper et al., 2017). Simsek et al. (2014) proceed to explain that individual groups between the ages of 9 and 15 years have different weight and other parameters. The measurements tend to influence the sports performance of adolescents of ages of 14 and 15 years and portray an enhanced performance while, on the other hand, their counterparts between the ages of 9 and 10 years exhibit minimal enhancement on performance. According to Joyner (2017), such factors as enduring performance, distance, and running speed during training are influenced by physiological aspects associated with sexual development during growth. In the age of 10 to 15 years, the childrens’ ability to undertake intense training, endurance, and athletic performance, varies significantly.

2.1.2 Hormone Production

Zakas et al. (1994) who studied boys with biological age of 10, 13 and 16 years old found that after 3 months of training, secreted GH and testosterone (T) in prepubertal subjects (10
At the age period of 13 to 16 years, however, GH and T secretory patterns undergo a remarkable change. They suggested that high intensity training can be a stimulus for increasing GH and T levels in puberty and adolescence. It has been reported by Malina (2006), that within a CA group, boys, who are advanced in maturation, tend to be on average taller, heavier and more powerful than boys later in maturation. In an earlier research, Miller et al. (1982) did not find any difference at the levels of GH during prepuberty, puberty and adolescence, while an other study by Zhang et al. (2000) suggested that mean concentration of GH from childhood to adolescence increased and reached a peak that is almost coincident with the time of PHV. Chahar (2014) points out that exercise has an imperative role in improving the endocrine system’s function. According to Richmond and Rogol (2016), exercise helps in enhancing neuro-endocrine control of the pituitary glands. Vigorous exercise also promotes the release of such hormones as T and GH. Children between the ages of 10-15 years should be encouraged to indulge in exercise to promote endocrine function. It has been reported by Hansen et al. (1999) that the players selected for the elite soccer group had a tendency for higher values of serum T, were taller, had lower values for skinfolds and body fat assessments, and were more advanced in maturity evaluated by testicular volume, compared to non-elite youth players.

2.1.3 Biological and Chronological Maturation – Tanner Stages

Cumming et al. (2018) describe biological maturation as a physiological change of body systems towards maturity, in regards to status, timing, and rate. Biological sexual maturation is indicated through the development of secondary sexual characteristics among both genders in the mentioned group. Malina et al. (2015) explain that the CA for the mentioned facet of biological maturation varies among various ages for children and young adolescents. However, menarchial
signs are a fundamental indicator of sexual maturity within CA groups of 11 to 15 years. Skeletal age is also an essential marker for maturation of the skeletal system among the mentioned group. Assessment of the skeletal age through such procedures - methods as Greulich Pyle (GP) and Tanner-Whitehouse (TW) helps to determine skeletal maturity concerning the CA of the children. Freitas et al. (2016) explain that skeletal maturation fundamentally influences the integration of excellent motor skills among young adolescents. Growth spurt characterised by increased height and muscle mass is also an important indicator of biological maturation. PHV is a CA indicator that sets a discrepancy on the level of growth spurt, height and maturity of young adolescents with those in the late stages of development, indicating peak height.

2.2 Anthropometric Characteristics

Anthropometry is described as the study of the measurement of the human body regarding the size of the bones, muscles and adipose tissue (Singh et al., 2017). The anthropometry consist of a variety of measurements of the human body; such as weight, height, body surface area (BSA), lean body mass (LBM), body mass index (BMI), skinfold thicknesses, circumferences, diameters and length measurements of the body segments (Fryar et al., 2012). In other words, anthropometry is the measurement of physical characteristics of the human body at different ages (Golden and Reilly, 2008). It includes taking accurate, highly standardized measurements so that the size and form of human body can be depicted objectively. Anthropometric dimensions, body composition and morphological characteristics are sensitive indicators of growth, progress and nutritional status of a population. The anthropometric measurements are used to evaluate the nutritional status in children and adults and are an effective and frequently performed child health screening procedure (Simko et al., 1995). The
anthropometric measurements of children are very important and are part of an overall clinical assessment. The data on anthropometric measurements of children reflect general health status, growth and development over time (Fryar et al., 2012).

2.3 Muscle Strength

It is important to note that the type of movements made while playing soccer requires one to have big muscles and strong lower extremities. Specifically, the musculature around the knee is important as it aids in injury prevention and equally enhances the proper functioning of the knee. However, there is little knowledge when it comes to the effect of specific training on peak torque (PT) of knee extensors and flexors in soccer among the developmental biological ages. Previously, in these athletes, the estimation of muscle strength of the low extremities had been derived from a vertical jump (Raven et al., 1976; Thomas and Reilly, 1979). During the last years, measurement of muscle strength is performed using the isokinetic dynamometer. However, little information is available on the isokinetic PT of Q and H muscles in young soccer players and especially in comparison with untrained young students of the same biological age. Some authors have suggested that H:Q strength ratios are good measures of normal knee function (Campbell and Glenn, 1982; Goslin and Charteris, 1979). Different values for the H:Q ratio have been reported (Campbell, 1979; Davies et al., 1981) and this ratio varies within different athletic groups (Stafford and Grana, 1984). Previous studies have reported moderate to high correlation between isokinetic muscle strength and various indices of soccer performance (Cabri et al., 1988; Poulmedis et al., 1988). Maximal strength refers to the highest force that can be performed during one maximum voluntary contraction, and it is considered very important for soccer performance (Brewer and Davis, 1991; Capranica et al., 1992; Davis et al., 1992;
Garganta et al., 1992). The evaluation of muscle strength of the lower extremities in soccer has been performed using the isokinetic dynamometer (Öberg et al., 1986; Zakas et al., 1995) and free weights (Wisloff et al., 1998). Coaches, trainers, and clinicians have identified isokinetic testing as a useful tool that provides critical information regarding muscle balance around the knee (Coombs and Garbutt, 2002). Öberg et al. (1986) showed a variation in concentric isokinetic PT of the Q and H muscles between the highest and the lowest soccer divisions. The conclusions made were that the high – level soccer players had greater strength as the intensity of training increased within higher playing divisions.

The strength of the lower limbs among players is of great concern. Specifically, the Q, H and triceps surae group must produce high forces useful for jumping, tackling, kicking, turning, changing pace and direction. The necessity of muscular strength in soccer is measured by putting together the measurements of muscular strength as well as the determinations of strength dependent skills in soccer such as kicking. Research posit that the combination of strength training and normal soccer training improves muscular strength and enhances kick performance (De Proft et al., 1988). However, the knee strength on its own does not dictate the final impact of the kick on the ball. Such has a relationship with hip muscles being among the key components of the soccer kick and the involvement of the antagonist muscles in the last part of a ball kick (Robertson and Mosher, 1983). The relationship that emerges between leg strength and kick performance shows that strength training is essential in improving kick performance of the soccer players. However, some studies found no relationship between kick performance and knee extensor strength as revealed by isokinetic dynamometry (McCrudden and Reilly, 1993; McLean and Tumilty, 1993).
2.3.1 Muscle Characteristics

H is a spindle (fusiform) muscle, which has a complex anatomy and functions four individual muscle portions, three of which span both the knee and hip joints (Askling et al., 2003). The H muscle group work by extending the hip joint and flexing the knee joint. They are also useful in different types of movement and equally in decelerating activities that involve eccentric contractions (Chumanov et al., 2007). Functionally, the co-activation of H, during concentric Q actions, significantly increased, however during concentric H muscle actions the co-activation of the Q did not significantly change (Wright et al., 2009). Sangnier and Tourny-Chollet (2007), investigated a group of soccer players during isokinetic endurance testing. The results indicated a divergence in fatigue resistance between Q and H. There was a significant decline in H strength that affected the balance of strength between the agonist and antagonist. Chen et al. (2011) demonstrated that the leg flexors inhibited greater muscle damage after an acute bout of eccentric exercise of similar volume and intensity as compared to the leg extensors. The H muscles have a relatively high proportion of fast twitch muscle fibers (type II) which are more involved with exercise of higher intensity and force production. Woodley and Mercer (2005) suggested that leg flexors consists of longer fibers (Narici et al., 1998; Wickiewicz et al., 1983, 1984), smaller total cross sectional area (CSA) and smaller muscle mass (Lieber, 2002). Due to these collective differences some authors (Chen et al., 2011, Garrett, 1990, Brockett et al., 2001) have suggested that the leg flexors are more vulnerable and more fatigable during long and eccentric exercise. On the other hand, the Q muscle fibers have a greater pennation angle, produce higher strength but imply lower speed. A long muscle, such as the H, contracts very fast and produces relatively high force (strength) even in high angular velocity.
2.3.2 Physiological Characteristics of Q and H Muscles in Children

The adult investigations of muscle biopsies can occur almost routinely but ethical consideration limits the technique on younger participants. This explains the limited muscle biopsy studies on younger people. Nevertheless, Dotan et al. (2012) report that fiber type distribution is the primary reason why children produce less force when compared to adults. The analysis of muscle morphology in elite soccer players has occurred on biopsies retrieved from m. vastus lateralis of the Q muscle or m. gastrocnemius. The results showed that the mean percentage of slow twitch (ST), type I, fibers ranged from 40% to 61% for m. vastus lateralis and from 49% to 60% for m. gastrocnemius (Bangsbo, 1994a). Both muscles gave a large variation on % ST fibres an indication of a unique distribution between slow twitch (ST) and fast twitch (FT). However, type II fibres are unnecessary when it comes to top-class players (Bangsbo, 1994a, b). Conversely, there was a variation when it comes to the observation of the number of type IIX fibers for elite players and non-elite players. The former had a smaller relative number compared to the latter. In another study, Metaxas et al. (2014) studied three groups of young soccer players of the ages 11, 13, and 15. The data of fibre type distribution on the dominant m. vastus lateralis indicated that the ST percentage distribution reduced with age as the player aged 15 had the lowest as compared to the other two groups. The researchers, therefore, made the conclusion that the high amount of the fast oxidative muscle fibers (IIA) witnessed among the oldest group as compared to the 11, and 13-year-olds is mainly ascribed to the different training programs and age factor as the activation of these fibres occurs during an intense exercise.
2.3.3 Architectural characteristics of Q and H muscle groups

Both muscle architecture and fibre type composition are important aspects when it comes to the determination of muscle function. Ideally, the muscle’s force potential is comparative to the physiological CSA (i.e. the number of sarcomeres that are in parallel) and to muscle fascicle length (i.e. the number of sarcomeres that are in series). What is more, the length of the muscle fascicle is a significant component of the muscle architecture and has an important functionality in muscle shortening velocity and power output (Lieber, 2002).

The biological maturation alteration in pennation angle has normally been muscle and site-specific. Although the pennation angle of the knee extensor muscle remains constant from childhood through to adulthood as described by O’Brien et al. (2010); the pennation angle of the m. gastrocnemius accelerates from birth before and stabilizes in later years as a result of the adolescent growth spurt (Kurihara et al., 2007). Lieber and Fridén (2000) claimed that an escalation in pennation angle as one matures is expected as it functions to expand the force–generating capabilities of a muscle. Resultantly, the longer fascicles lead to an enhanced capability to generate force at higher velocities (Blazevich 2006). Predictably, Lieber (2002) affirms that muscles appear into two main morphological types. The first type is the fusiform muscles (as H), where fibres appear to be parallel to the line of action of the muscle. The second type is pennate muscles (as Q), where fibres insert into tendons at an angle to the line of action of the muscle. When comparing the two types of muscles, the pennate muscles has a number of sarcomeres in series that enhances the force development or shortening action resulting in a shorter fascicle length when compared to the fusiform muscles. Besides, the fibres are placed at an angle to the line of force generation, with shorter fibre length and relatively high CSA, an aspect that advocates force generation, instead of the speed of movement (Abe et al., 1998).
2.3.4 Familiarisation of strength measurements

Familiarisation with the equipment and the type of exercise has raised a significant debate thus drawing significant attention from various scientists. According to research, there exists a period of a learning effect when an individual is adapting to a new type of movement (Erim et al., 1996). The reasons for this is that there are different neural mechanisms involved. As a result, the preliminary results often overestimate the measured value as shown by Enoka (1988). With the purpose of avoiding misinterpretation, there should be an implementation of familiarisation sessions to ensure the convergence of the outcome to achieve a better approximation of the real value. It is worth noting that participants who have never had a previous experience would need more familiarisation sessions than experienced participants (Newton et al., 2008). In addition, age is a significant factor that affects the number of familiarisation sessions required (Ploutz-Snyder and Giamis, 2001; Wallerstein et al., 2010). As pertains to the size of the muscular group involved, the large muscle groups require longer number of sessions and vice versa (Ritti-Dias et al., 2005). An appreciation of the relative isokinetic torque values would provide even more valid data for the approximation of the subjects’ real values (Harding et al., 1988). Finally, it is essential that the methodological approach is appropriate and informative, regarding the testing procedure (position, testing protocol and feedback); nevertheless it is crucial for the researchers to know, not only the statistical difference between the muscle groups, subjects and ages, but also the absolute and relative differences in Nm, in order to decide if they are acceptable for specific populations.
2.3.5 Imbalances in strength between H and Q muscles

Lower limb isokinetic muscle strength tests have mainly focused on the assessment of the knee joint. One primary goal in the assessment of muscular strength and power is to identify gross muscle weakness or functional imbalances, between legs and also between agonistic and antagonistic muscle groups of the same leg (i.e. between knee flexor and knee extensor muscle groups). Previous reports in adults suggested that conventional H:Q strength ratio should be at least 0.6, namely H are 60% as strong as the Q (Coombs et al. 2002; Holcomb et al. 2007), for preventing H and/or knee related injuries (Sangnier and Tourny-Chollet, 2007; Yeung et al., 2009; Coombs et al., 2002). Q and H are two muscle groups with different function. It has been reported that co-activation of H muscles significantly increases during concentric Q muscles contractions. However, this is not the case for the opposite, as co-activation of Q muscles does not change during concentric H muscle contractions (Wright et al., 2009).

2.3.6 Muscle and blood lactate concentration in children

An earlier study conducted by Eriksson and Saltin (1974) confirmed that muscle phosphocreatine (PCr) and glycogen stores, gradually decline after once conducts an intense exercise that results in glycogen depletion. This outcome is three times greater in 15-year-olds than in 11 years old. However, as glycogen decreases there was a corresponding increase in muscle lactate production seen to be higher in older boys. The conclusion made by most studies is that unlike adults, children have a well-developed capacity for oxidative metabolism during exercise, which may be a hindrance for anaerobic metabolism. Previous studies have shown that the lower blood lactate concentration during maximal exercise in children, in comparison with adults, is due to a lower muscle blood lactate concentration. Another reason for the low
concentration in children, compared to adults, is the low concentration of the phosphofructokinase enzyme. The concentration increases as age increases. The studies also showed a minimal connection between maximal muscle lactate and testicular volume in 13 years old boys, signifying an influence on maximal lactate production (Eriksson et al., 1971a, b; Eriksson and Myrhage, 1972).

2.4 Physiological demands

2.4.1 Energy production

The main source of energy for the muscular contraction is carbohydrate and fat from the food consumed. Energy can be supplied to the contractile elements of skeletal muscle anaerobically from adenosine triphosphate (ATP), creatine-phosphate (CP) and from degradation of glycogen to lactate. The phosphagen system, ATP and CP, provides energy rapidly when an urgent need arises for immediate use of energy. ATP in skeletal muscle cells can be restored by the condensation of two molecules of adenosine diphosphate (ADP) to one ATP and one adenosine monophosphate (AMP). If muscular contraction should continue for more than a few seconds, the level of ATP must continually be restored. Replenishment of ATP can take place via the oxidative or the glycolytic pathways. During maximal exercise of short duration all three sources of anaerobic energy (ATP, CP, and glycogen) are used. Hultman et al. (1967) observed a linear relationship between work intensity and the reduction in CP. ATP declined during moderate workloads, but no further reduction with increasing work load was observed. The
glycolytic system provides energy for short-term needs. It is a metabolic pathway, known as anaerobic glycolysis, where glucose is degraded into pyruvate, which is turned to lactate. This system predominates during muscular efforts of high intensity that last up to 1 minute.

The oxidative system provides energy over an extended period of time. Under aerobic conditions, pyruvate becomes acetyl-coenzyme A. This coenzyme can be formed from the breakdown of amino acids and fatty acids. All these transformations is produced in the mitochondrion that exist inside the muscle cell by using oxygen drawn from the blood. The substrates for these reactions result from the processes of glycolysis, utilisation of carbohydrate, catabolism of fat and a lesser extent, proteins. The aerobic energy produces large sums of ATP.

While playing soccer, players engage in various activities and the intensity varies. For instance, it ranges from standing still to maximal running. The physiological demands of soccer require players to complete several aspects of fitness to include aerobic and anaerobic energy. The anaerobic energy occurs because of the breakdown of adenosine triphosphate (ATP) stored within the muscle or produced either by splitting creatine phosphate (CP) or by degrading carbohydrate to pyruvate hence the formation of lactate. The rate at which ATP is produced during exercise relies on the intensity of the exercise.

2.4.2 Aerobic energy production

The most definite way to get information about aerobic energy spending during a match is by recording the heart rate (HR) continuously to estimate energy expenditure from HR – \( \dot{V}O_2 \) relation determined in the laboratory. According to Seliger (1968), the mean HR values during a match was 165 beats min\(^{-1}\) (80% of maximal HR (HR\(_{max}\))) and 175 beats min\(^{-1}\) (93% HR\(_{max}\)) similar to findings by Agnevik (1970). On the other hand, Smidlaka (1978) showed that HR for
Russian players exceeded 85% of HR$_{\text{max}}$ (171 beats min$^{-1}$) for 57% of the playing period while Reilly (2003), found an average HR of 157 beats min$^{-1}$ on English participating in friendly matches.

### 2.4.3 Aerobic fitness

Different aerobic factors work well when it comes to determining the ability to sustain a high work rate for 90 minutes. Among these aerobic factors includes both aerobic power ($\dot{V}O_2_{\text{max}}$) and a high functional utilization of $\dot{V}O_2_{\text{max}}$. The aforementioned Midfield players of English League showed the highest values for aerobic power. Alternatively, the central defenders recorded relatively lower values as compared to the other outfield players. However, Reilly (1990), showed the fullbacks and strikers to have had intermediate values. The significant association of $\dot{V}O_2_{\text{max}}$ and the total distance covered in a match ($r=0.67$) shows there is a need for midfield players who act as a link between defence and offence to adopt a high work rate and a high aerobic fitness level.

### 2.4.4 Physiological profile

The importance of the phosphagen system (CP + ATP) in maintaining speed during repeated bouts, when exercise periods are short, as in soccer (Bogdanis et al., 1996; Casey et al., 1996; Gaitanos et al., 1993; Hitchcock, 1989). For instance, during a short maximal bout of 6-seconds, phosphocreatine (PCr) contributes about 50% of the anaerobic energy utilized, after, which the glycolysis process dominates. However, repeated bouts lead to an increase in the PCr contribution (Little and Williams, 2007).
2.5 Limitations of previous research and Rationale for current study

To the best of our knowledge, most of the studies have investigated the effects of training during childhood (prepubertal and pubertal) on the isokinetic muscle strength and aerobic power between children of the same CA. Hence, the results reported raise concerns regarding the effects of training, because they are difficult to separate from the effects of growth. Additionally, there are objections in the methodological approach followed by the researchers, since they did not provide detailed information regarding the familiarization sessions, the testing procedure and the feedback. Furthermore, the statistical analysis performed by most of the scientists was independent sample T-test analysis and ANOVA. However, as Atkinson and Nevill (1998) suggested, these methods have limitations as they may mask the “real” differences. Moreover, it is crucial for the researchers and coaches to know the absolute differences between sessions (in Nm) in order to decide if they are acceptable for specific applications. For these reasons, it is essential to make a thorough investigation considering the biological age, comprising a range of angular velocities, in order to examine and analyze the differences in absolute and relative PT as well as the anthropometric and physiological parameters between adolescents.
2.6 Purpose of the study

The purpose of the present study was to examine and compare the physical and physiological characteristics and muscle strength between young soccer players and untrained adolescents, who participated only at the physical activities in school, of the same biological age (12, 14 and 16 years). More specifically, the aims of this study were:

- To examine the anthropometric characteristics; such as weight, height, BSA, LBM, BMI.
- To evaluate the absolute and relative $\dot{V}O_2_{\text{max}}$, HR_{rest}, HR_{max} BLa_{max} and BP_{rest}.
- To measure and investigate the concentric isokinetic muscle strength of knee extensors and knee flexors at a range of angular velocities along with:
  - The changes (%) in absolute and relative PT values of H and Q at different angular velocities among different age groups for trained and untrained adolescents.
  - The absolute and relative PT values of the H and Q, their quantitative differences in Nm and their percent decrease from slower to faster angular velocity.
  - The H:Q strength ratio across angular velocities and age groups for trained and untrained adolescents.
2.7 Hypotheses

Several research hypotheses were employed for the purpose of this study:

$H_a$: Young soccer players will have higher absolute and relative muscle strength than untrained adolescents.

$H_a$: The strength ratio of $H:Q$ will be higher in young soccer players, in comparison with the young adolescents of the same age.

$H_a$: PT values of knee extensors and knee flexors will significantly increase with age, whereas the ratio of $H:Q$ will be different among ages.

$H_a$: $VO_2\text{max}$ will be different between young soccer players and untrained healthy adolescents of the same age.
3. Materials and Methods

3.1 Participants

A total of one hundred and twenty six (n=126) young boys throughout the developmental ages of 12 (n=44), 14 (n=38) and 16 (n=44) volunteered to participate in this study. The study was performed in accordance with the local university Ethics Committee guidelines and ethical standards of the Sports Medicine research (Appendix 8). The participants were divided according to their biological age in three age groups and each group was divided into two sub-groups: a) soccer players as trained group (12 years, n=22, training experience= 4.9±2.2 years; 14 years, n=20, training experience= 6.2±1.8 years; 16 years, n=22, training experience= 8.7±1.6 years), b) untrained boys (12 years, n=22; 14 years, n=18; 16 years, n=22). The physical characteristics of the adolescents are shown in Table 1. All participants and their parents were informed of the nature, purpose, procedures, potential discomfort, risks and benefits involved in the study before giving their voluntary written consent for participation (Appendix 2 – 4). All participants completed a questionnaire that included their relevant medical and physical history. None of the participants reported musculoskeletal injuries of the lower limbs that would prevent them from performing maximal exercise (Pescatello, 2013) (Appendix 5 – 6). The exclusion criteria were: recent history of muscle injury of the lower limbs, present complaint of thigh and leg pain and any other medical problems contraindicated for experimental testing. Also, overweight boys were excluded from the untrained group. None of the participants had been doing progressive resistive exercise 24 hours before the testing as well as their sleep pattern was sufficient (6 – 8 hours) in order to arrive to the laboratory in a rested condition (Blacker et al., 2010) (Appendix 3, 7). All players in the study came from the same club and from three associate soccer
academies, which followed a specific training program, while the untrained came from four different schools of the same region. The untrained group participated only in their normal Physical Education program in their school and did not take part in any other sport activities. The trained group participated both in their schools Physical Education program and in the national championship competition games. They were a highly selective group with regard to skills, performance, size, anthropometric characteristics and physical condition. Pubertal staging, by examination of pubic hair, penile and testicular development was performed by an experienced paediatric physician, experienced in the assessment of secondary sex characteristics, state of pubic hair based on the criteria of Tanner (1962). The participants visited the laboratory on several occasions and were accustomed to both the laboratory environment and equipment and were informed from the investigator about the experimental procedures. To reduce systematic measurement errors, the participants were supplied with standardised verbal instructions adapted to young population. All measurements were undertaken by the same investigator (Athanasios Mandroukas) in order to prevent intertester variability.

3.1.1 Group of youth soccer players

All soccer players in the current study came from the same club and followed a consistent training program across the age groups. Training in prepubertal and pubertal age (12-16 years old), in general, aims at creating versatile players by developing physical performance through specific exercises tailored to the spot of soccer and age level. None of the participants followed a personalised weight training program designed by their academy.
3.1.1.1 Training Program

More specifically, the soccer players were recruited based on the modern model of development that occurs at young ages and applies the results of the laboratory testing in training practice, in order to improve the sport performance of young soccer players. The duration of the training season for all athletes was 44 weeks per year. In particular, the 12 years old subjects systematically performed 3 training sessions per week of 75 min each, while the 14 and 16-year-old athletes participated in 4 and 5 training sessions of 90 min each per week, respectively. Furthermore, all players had 1 additional specific personal training session every 15 days for individual improvement in certain skills. In total, the annual amount of training sessions for the 12, 14 and 16 years of age groups were 130, 170 and 220, respectively. Moreover, all players competed in one game per week throughout the season. The training protocol was based on the normal technical-tactical and physiological elements of soccer indicated for improvement according to developmental age.

3.1.2 Untrained group

The school Physical Education program consisted of 2-3 training sessions per week for all age groups and every session lasted 40 minutes. The content of this program consisted of ball games (e.g. soccer, basketball, volleyball, handball and some calisthenics). The intensity was estimated by telemetry (Sport Tester, Finland). Both the duration and the intensity of the training in this group were much less than the specific training program of the soccer players.
3.2 Measurements

3.2.1 Anthropometric measurements

The standing height was measured to the nearest 0.5 cm using a stadiometer, and the body weight was measured using an electronic digital scale (Seca 220e, Hamburg, Germany). All participants underwent anthropometric examinations, including BSA, LBM and BMI.

3.2.1.1 BSA

The body surface area was calculated by the following formulae from Mosteller (1987).

\[
BSA \ (m^2) = \sqrt{\frac{\text{height (cm)} \times \text{weight (kg)}}{3600}}
\]

3.2.1.2 LBM

The lean body mass was calculated using equations of Cunningham (1980).

\[
\text{Lean body mass (LBM)} = (1.10 \times \text{weight (kg)}) - 128 \ (\text{weight}^2 / (100 \times \text{height (m)})^2)
\]

3.2.1.3 BMI

Body mass index was calculated by the following formulae

\[
\text{BMI (Kg/m}^2) = (\text{Body mass in Kg}) / (\text{Stature in Meters})^2 \ (\text{Meltzer et al., 1988})
\]

3.2.2 Blood Pressure

Arterial BP and resting HR were measured in a resting state, with the participants in the supine position with their legs uncrossed, and the middle of the cuff at the level of the right atrium of their heart. Measurements were performed in a quiet room under standardised conditions, and between 9:00 and 10:00 h to avoid diurnal variations (Schmidt et al., 2015). The
participants rested for at least 5 minutes in order to acclimate to the new environment and to allow their blood pressure to normalize. An automatic BP monitor (M7, Omron, Vernon Hills, Illinois, USA) was used with a cuff adjusted to the arm size as appropriate. Resting HR was measured simultaneously by the automatic PB monitor.

3.2.3 Isokinetic Strength Testing

3.2.3.1 Warm-up

Prior to each testing session, participants followed a standardised warm-up on a cycle ergometer (Monark 839, Varberg, Sweden) for 5 min with low resistance at 60 rev/min, prior to all strength measurements. The seat and height of the cycle ergometer was adjusted so that the knee was fully extended when the pedal was in the lowest position. This exercise was followed by a 5 min partial passive stretching of the knee flexors and extensors according to Mandroukas et al. (2014) and the unilateral concentric muscle strength of the dominant leg was measured on the isokinetic dynamometer.

3.2.3.2 Testing protocol

The strength of knee flexors and extensors in the dominant leg were measured using an isokinetic dynamometer Cybex II (CSMI, Humac Norm, Cybex II). For each angular velocity, peak isokinetic torque was recorded simultaneously, and the torque generated by the limb weight and the dynamometer arm was extracted from the obtained data. The participant was then seated on the dynamometer in an adjustable chair; the upper body was stabilized with straps secured diagonally across the chest, around the hips and thighs, to prevent any extraneous joint movement. The knee tested, was positioned at 90° of flexion (0° corresponding to fully extended knee) to align the axis of the dynamometer lever arm with the distal point of the lateral femoral
condyle. The length of the lever arm was individually determined, and the resistance pad placed at 5 cm above the malleoli. The non-tested leg was hanging freely. Knee extension started when the knee was positioned at 90° of flexion, while the knee flexion started when the knee was in full extension (0°). Prior to maximal testing, a series of submaximal concentric contractions were performed. Subjects were instructed to cross their arms over their chest and to kick the leg as hard and as fast as they could through a complete ROM. Three repetitions were carried out at each angular velocity and the best PT value of the three trials was used. A 30-second rest period was taken between each trial and a 60-second rest period was taken between each velocity measurement. During these procedures participants always performed maximal voluntary contractions, in which verbal encouragement was given to the participants throughout the contractions. Maximal isokinetic strength was recorded as the torque of the H and Q muscles throughout the whole ROM at angular velocities of 60, 180 and 300°·sec\(^{-1}\). The concentric strength ratio between the knee flexors and the knee extensors (H:Q ratio) was expressed as the ratio between the peak values at each velocity. The conventional H:Q ratio was calculated by dividing each participant’s highest concentric PT leg flexion by the highest concentric PT leg extension.

3.2.4 Determination of maximum oxygen uptake

Maximal oxygen uptake (\(\dot{V}O_2\text{max}\)) was performed on a motorised treadmill (Pulsar, H/P Cosmos, Germany) after a five-minute warm-up and stretching of the lower limbs. \(\dot{V}O_2\text{max}\) was determined using an uphill incremental continuous treadmill running test to exhaustion. The initial grade and speed were set at 0% at 6 km/h for the 12 years old and at 8 km/h for the 14 and 16 years old, for 4 minutes, and followed by stepwise increase in speed of 1 km/h every minute
per stage with 2% stable grade until exhaustion. The oxygen uptake, determined by means of absolute \( \text{ml} \cdot \text{min}^{-1} \) and relative values adjusted to body weight \( \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \), as well as the cardiorespiratory indices were measured via an ergospirometric device based on breath by breath automated pulmonary/metabolic gas exchange system (Oxycon Pro-Jaeger, Würzburg, Germany) using a tight face mask specially designed for children. The HR was recorded continuously using a Polar HR monitor (Polar Electro, Oy, Kempele, Finland) connected to the ergospirometric device. Subsequently, the following additional cardiorespiratory indices were determined during the test: the exercise duration; the respiratory exchange ratio (RER); and the maximal heart rate \( \text{HR}_{\text{max}} \). \( \dot{V}_\text{O}_2\text{max} \) was assumed when three of the four following criteria were met: a) the HR during the last minute exceeded 95% of the expected maximal HR predicted 220-age; b) a RER \( \text{VCO}_2/\dot{V}_\text{O}_2 \) at or higher than 1.1 was reached; c) \( \dot{V}_\text{O}_2 \) reached a plateau and/or signs of subjective exhaustion were present and the subject was unable to continue running, despite verbal encouragement; d) level of concentration of blood lactate higher than 6mmol·l\(^{-1} \) (Chamari et al., 2004, 2005). Blood samples were obtained from the hand warmed fingertip and the concentration of blood lactate was determined in the 5\(^{th} \) minute of recovery using a lactate photometer analyser (Accusport, Boebringer Manheim, Germany). The rating of perceived exertion (RPE) was obtained during the maximal exercise by use of a general Borg scale (Borg, 1990).
3.3 Data / Statistical Analysis

Data for strength measurements were taken from Cybex II (CSMI, Humac Norm, Cybex II) software package. The factors for the evaluation of strength performance were Absolute PT (APT) and Relative PT (RPT). APT defined as the best value from all repetitions and RPT as the best value expressed as a percentage of body weight of all repetitions, for every type of movement and velocity. Whilst both APT and RPT values were calculated, most attention has been made in the APT values because this is what usually researchers used in previous studies. However, whenever is needed the RPT values have also been added, but for full RPT Values please see Appendices.

Statistical analysis was undertaken using SPSS V.22.0 (SPSS Inc., Chicago, Illinois, USA) and Microsoft Excel 2013 (Microsoft Corp., Redmont, Washington, USA). Initially, descriptive statistics were used to calculate means and standard deviations for the testing sessions, for all groups. A two-way analysis of variance (ANOVA) was used to compare the effect of two categorical independent variables. Multiple comparisons with Bonferroni Post-hoc tests were used to determine which groups in the ANOVA differ from each other, particular whether there were significant differences between trained and untrained adolescents and between age groups. Level of significance was set at p<0.05. Also, for the strength measurements, the absolute differences (Nm and %) were calculated together with the standard deviation between muscle groups; age groups and angular velocities, in order to have an appreciation of absolute changes between trained and untrained adolescents.
4. Results

The physical and anthropometric characteristics between trained and untrained adolescents in different age groups are shown in Table 1.

Table 1. Physical and anthropometric characteristics between trained and untrained adolescents in different age groups (mean±SD)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>12 years (n=44)</th>
<th>14 years (n=38)</th>
<th>16 years (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Training age (yrs)</td>
<td>4.9±2.2</td>
<td>6.2±1.8</td>
<td>8.7±1.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.4±12.8</td>
<td>152.4±7.4***</td>
<td>166.5±8.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52.5±13.4</td>
<td>52.0±13.0</td>
<td>56.9±9.5</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.5±0.3</td>
<td>1.5±0.2</td>
<td>1.6±0.2</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>44.1±9.8</td>
<td>42.0±7.7</td>
<td>47.6±6.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.8±2.5</td>
<td>22.1±3.7**</td>
<td>20.5±2.4</td>
</tr>
</tbody>
</table>

*** U12 trained vs U12 untrained, p<0.001; ** U12 trained vs U12 untrained, p<0.01
(Multiple comparisons: Bonferroni Post-hoc).
There were no significant differences in height (cm) between trained and untrained boys at the ages of 14 and 16 years, however the 12-year-old trained boys were significantly taller in comparison with the untrained boys of the same age (p<0.001) (Table 1). Also, the 12 years untrained boys were shorter in comparison with the 14 and 16-year-old untrained (p<0.001), but there were no significant differences between the 14 and 16 untrained boys (Figure 1). For the trained group, there were significant differences between the 12 and 16-year-old boys and between 14 and 16-year-olds (p<0.001). However, there were no significant differences between the 12 and 14-year-old trained adolescents (Figure 1).

![Graph showing height (cm) among different age groups of trained and untrained adolescents](image)

Figure 1. Height (cm) among different age groups of trained and untrained adolescents (mean ± SD).

+++ U12 untrained vs U14 untrained, p<0.001; ††† U12 untrained vs U16 untrained, p<0.001; ‡‡‡ U12 trained vs U16 trained, p<0.001; ††† U14 trained vs U16 trained, p<0.001.

The body weight (kg) did not differ between trained and untrained adolescents at all ages (Table 1) and between 12 and 14 years, both in trained and untrained adolescents. However,
there were significant differences between the 14 and 16-year-old boys, both in trained and untrained adolescents (p<0.01 and p<0.05 respectively). Also, significant differences were observed between the 12 and 16 years old trained and untrained boys (p<0.001) (Figure 2).

Figure 2. Body weight (kg) among different age groups of trained and untrained adolescents (mean ± SD).

!!! U12 untrained vs U16 untrained, p<0.001; * U14 untrained vs U16 untrained, p<0.05; ** U12 trained vs U16 trained, p<0.001; §§ U14 trained vs U16 trained, p<0.01.

Furthermore, there were no significant differences between trained and untrained adolescents in all age groups regarding the BSA (m$^2$) and the LBM (kg) (Table 1). The results were similar for the BSA (m$^2$) (Figure 3) and LBM (kg) (Figure 4) among the different age groups of trained and untrained. More specifically, significant differences were found for the untrained group between the 12 and 14 years (p<0.01); 14 and 16 years (p<0.05) and 12 and 16 years (p<0.001). For the trained group, significant differences were observed between the 14 and
16-year-olds and between 12 and 16 years (p<0.001); nonetheless no significant differences were found between the 12 and 14 years old trained adolescents (Figure 3, 4).

Figure 3. BSA (m²) among different age groups of trained and untrained adolescents (mean ± SD).

†† U12 untrained vs U14 untrained, p<0.01; !!! U12 untrained vs U16 untrained, p<0.001; † U14 untrained vs U16 untrained, p<0.05; ☢ ☢ ☢ U12 trained vs U16 trained, p<0.001; §§§ U14 trained vs U16 trained, p<0.001.
Figure 4. LBM (kg) among different age groups of trained and untrained adolescents (mean ± SD).

†† U12 untrained vs U14 untrained, p<0.01; ‼‼ U12 untrained vs U16 untrained, p<0.001; ‼️ U14 untrained vs U16 untrained, p<0.05; ℹ️ U12 trained vs U16 trained, p<0.001; §§§ U14 trained vs U16 trained, p<0.001.

The BMI (kg/m²) was significantly different at 12 years between trained and untrained boys (p<0.01) but there were no significant differences in 14 and 16 age groups (Table 1). Moreover, there were no significant differences for both trained and untrained adolescents among the ages (12, 14 and 16) (Figure 5).
Figure 5. BMI (kg/m$^2$) among different age groups of trained and untrained adolescents (mean ± SD).

The physiological characteristics and the results of the cardiopulmonary testing between trained and untrained adolescents in different age groups are shown in Table 2.
Table 2. Physical and physiological characteristics between trained and untrained adolescents in different age groups (mean±SD).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>12 years (n=44)</th>
<th>14 years (n=38)</th>
<th>16 years (n=44)</th>
<th>Trained</th>
<th>Untrained</th>
<th>Trained</th>
<th>Untrained</th>
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<td>(n=18)</td>
<td>(n=22)</td>
<td>(n=22)</td>
<td>(n=22)</td>
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<td></td>
</tr>
<tr>
<td>HR&lt;sub&gt;rest&lt;/sub&gt; (b·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>69.9 ± 9.6 ***</td>
<td>78.2 ± 7.3 ***</td>
<td>65.0 ± 3.0 ###</td>
<td>73.1 ± 9.0</td>
<td>9.0</td>
<td>61.2 ± 4.8</td>
<td>4.8</td>
<td>63.8 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>sBP&lt;sub&gt;rest&lt;/sub&gt; (mmHg)</td>
<td>118.4 ± 7.1 *</td>
<td>122.7 ± 5.7 #</td>
<td>112.9 ± 5.0 ¥</td>
<td>118.6 ± 6.8</td>
<td>6.8</td>
<td>119.5 ± 9.4 §</td>
<td>6.4</td>
<td>120.2 ± 8.2</td>
<td></td>
</tr>
</tbody>
</table>
| dBP<sub>rest</sub> (mmHg) | 63.0 ± 7.4 *** | 69.5 ± 6.7 *** | 63.6 ± 5.4 †† | 62.2 ± 4.3 | 5.4 | 61.4 ± 6.4 | 6.4 | 62.5 ± 6.6 | !!!!
| VO<sub>2</sub>max (ml·min<sup>-1</sup>) | 2978.4 ± 803.1 *** | 2112.3 ± 337.6 | 3607.7 ± 343.9 ### | 2908.3 ± 393.5 | 803.1 *** | 337.6 | 548.8 $$$ | 192.1 |
| VO<sub>2</sub>max (ml·kg<sup>-1</sup>·min<sup>-1</sup>) | 57.0 ± 5.9 *** | 41.4 ± 3.9 | 65.1 ± 12.8 ### | 49.5 ± 4.9 | 3.9 | 57.9 ± 7.1 $$$ | 4.9 | 43.9 ± 5.0 | |
| HR<sub>max</sub> (b·min<sup>-1</sup>) | 198.3 ± 6.9 | 195.0 ± 4.3 | 197.8 ± 4.5 | 198.0 ± 5.6 | ± 4.5 | 190.8 ± 6.9 $ | ± 5.6 | 144.3 | |
| BLa<sub>max</sub> (mmol·l<sup>-1</sup>) | 8.4 ± 0.7 | 8.5 ± 0.6 | 8.7 ± 1.8 ### | 6.4 ± 1.2 | 1.8 ### | 9.4 ± 1.3 $ | 1.2 | 8.7 ± 0.7 | |
| Respiratory exchange ratio (RER) | 1.1 ± 0.0 * | 1.2 ± 0.1 | 1.2 ± 0.2 ### | 1.0 ± 0.1 | 0.1 | 1.1 ± 0.1 | 0.1 | 1.2 ± 0.1 | |

*** U12 trained vs U12 untrained, p<0.001; * U12 trained vs U12 untrained, p<0.05; U14 trained vs U14 untrained, p<0.001; # U14 trained vs U14 untrained, p<0.05; $$$ U16 trained vs U16 untrained, p<0.001; ¥ U12 trained vs U14 untrained, p<0.05; $ U16 trained vs U16 untrained, p<0.05; ¥ U14 trained vs U14 trained, p<0.05; †† U12 untrained vs U14 untrained, p<0.01; !!!! U12 untrained vs U16 untrained, p<0.001 (Multiple comparisons: Bonferroni Post-hoc).
HR_{rest} in 12 and 14 years old trained adolescents was significantly lower (p<0.001) compared to untrained adolescents. No significant differences were found in 16-year-olds between trained and untrained adolescents (Table 2). Also, the 16 years untrained boys had significantly lower resting HR values in comparison with the 14 and 12 years old untrained (p<0.001); however, there were no significant difference between the 12 and 14 untrained adolescents (Figure 6). For the trained group there were no significant differences between the 12 and 14 years old trained adolescents as well as between the 14 and 16 trained. The only significant difference for that group were found between the 12 and 16 years old trained boys (Figure 6).

![Figure 6. HR_{rest} (b min^{-1}) among different age groups of trained and untrained adolescents (mean ± SD).](image)

!!! U12 untrained vs U16 untrained, p<0.001; ینین U14 untrained vs U16 untrained, p<0.001; ⊶造型 U12 trained vs U16 trained, p<0.001.
The systolic blood pressure in rest (sBP\text{rest}) was significantly lower in trained adolescents compared to the untrained in all age groups (p<0.05); as well as between 12 years and 14 years trained adolescents (p<0.05). No significant differences were found for the trained group between the 14 and 16 and between 12 and 16 trained boys. Also, for the untrained group no significant differences were observed among all age groups (Table 2).

Diastolic blood pressure in rest (dBP\text{rest}) was significantly lower in the 12 years old trained adolescents in comparison with the untrained (p<0.001); nonetheless, no significant differences were observed between trained and untrained for the 14 and 16 years old boys, as well as for the trained group among all ages. For the untrained group, the 12 years old boys had significantly lower values in comparison with the 14 (p<0.01) and 16 (p<0.001) years old. No significant differences were observed between the 14 and 16 years old untrained adolescents (Table 2).

The $\dot{V}O_{2,max}$ both in absolute (ml·min$^{-1}$) and relative (ml·kg$^{-1}$·min$^{-1}$) values was significantly higher in the trained adolescents compared to untrained in all ages (p<0.001) (Table 2). For the untrained group, the 12-year-old boys had significantly lower values in comparison with the 14-year-olds, both in absolute (p<0.001) (Figure 7a) and relative (p<0.01) (Figure 7b) values. Also, the 14 years old untrained boys had significantly lower relative values in comparison with the untrained 16 years (p<0.05) (Figure 7b); however the absolute values did not differ between these ages. Similarly, the 12 years old untrained adolescents had lower absolute values in comparison with the 16 years (p<0.001) (Figure 7a); nonetheless, no significant differences were observed in the relative values. For the trained group, similar patterns were observed. More specifically, the 12 years old trained boys had significantly lower absolute (p<0.001) (Figure 7a) and relative (p<0.01) (Figure 7b) values, in comparison with the
14 years old trained. Additionally, the 14 years old trained adolescents presented significantly lower relative $\dot{V}O_2\text{max}$ values in comparison with the 16 years old trained boys ($p<0.01$) (Figure 7b), but the absolute $\dot{V}O_2\text{max}$ values were not significantly different among these age groups. Finally, the 12 years old trained boys presented significantly lower absolute values in comparison to the 16 years trained group ($p<0.001$) (Figure 7b); but no significant differences were found in the relative values.

(a)
Figure 7. Absolute (a) and Relative (b) maximal aerobic power among different age groups of trained and untrained adolescents during the maximal running test. Values represent mean ± SD.

†† U12 untrained vs U14 untrained, p<0.01; ††† U12 untrained vs U14 untrained, p<0.001; ‼‼ U12 untrained vs U16 untrained, p<0.001; ‼ U14 untrained vs U16 untrained, p<0.05; ″″″ U12 trained vs U14 trained, p<0.001; ″″ U12 trained vs U14 trained, p<0.01; ♂♂♂ U12 trained vs U16 trained, p<0.001; §§ U14 trained vs U16 trained, p<0.01.

Maximal HR (HR_{max}) did not differ significantly between trained and untrained at 12 and 14 years old adolescents, however significant difference were found at the age group of 16 years (p<0.05) (Table 1). Furthermore, there were no significant differences for the untrained group in all ages as well as for the trained group between 12 and 14 years old trained adolescents. Also, for the trained group there were significant differences for the 16 years old boys in comparison with the 12 and 14 years old (p<0.001) (Figure 8).
Figure 8. \(HR_{\text{max}}\) (b·min\(^{-1}\)) among different age groups of trained and untrained adolescents (mean ± SD).

©©© U12 trained vs U16 trained, \(p<0.001\); §§§ U14 trained vs U16 trained, \(p<0.001\).

The maximal blood lactate concentration (\(\text{BL}_{\text{a max}}\)) was significantly different in 14 and 16 years for trained and untrained adolescents (\(p<0.001\) and \(p<0.05\) respectively), however no significant differences were found between the 12 years old trained and untrained boys (Table 2). For the untrained group, the \(\text{BL}_{\text{a max}}\) values for the 14 years old boys were significantly lower in comparison to the 12 (\(p<0.001\)) and the 16 (\(p<0.05\)) years old untrained adolescents, but there was no significant difference between untrained boys of 12 and 16 years (Figure 9). For the trained group, there were no significant differences among the different age groups, except from the 12 years old untrained boys, compared to the 16 age group (\(p<0.05\)) (Figure 9).
**Figure 9.** BLa\textsubscript{max} (mmol\textperiodcentered l\textsuperscript{-1}) among different age groups of trained and untrained adolescents (mean ± SD).

††† U12 untrained vs U14 untrained, p<0.001; ⁄⁄⁄ U14 untrained vs U16 untrained, p<0.001; © U12 trained vs U16 trained, p<0.05.

RER was significantly different between 12 years old trained and untrained boys (p<0.05) as well as for the 14 age group (p<0.001). Conversely, no significant difference was found for the 16-year-old adolescents (Table 2). For the untrained group, there were significant differences for the 14 years old boys, in comparison to the 12 and the 16 years old untrained adolescents (p<0.01), but no significant differences were observed between the 12 and 16 untrained age groups (Figure 10). In addition, no significant differences were found for the trained boys among all age groups.
Figure 10. RER values among different age groups of trained and untrained adolescents (mean ± SD).

†† U12 untrained vs U14 untrained, p<0.01; ▼▼ U14 untrained vs U16 untrained, p<0.01.

The absolute isokinetic muscle strength of knee extensors and flexors between trained and untrained adolescents in different age groups and angular velocities are presented in Figures 11, 12, and 13. Furthermore, the changes (%) and the differences (Nm) in APT values of Q and H at different angular velocities, among different age groups for trained and untrained, are presented in Figures 14, 15 and 16. Moreover, the APT values of Q and H, the differences in Nm, the percent decrease between the angular velocities and the H:Q strength ratio across angular velocities and age groups for trained and untrained adolescents, are presented in Figure 17. Significant differences were found at all angular velocities between the trained and untrained adolescents at the age group of 12 years old (p<0.001) and 16 years old (p<0.001) for both Q and H muscles. However, it is worth noting that no significant differences were found between
trained and untrained 14 years old adolescents at all angular velocities (i.e. 60, 180 and 300°·s⁻¹) both in knee extensors and flexors (Figures 11, 13 and 15).

![Figure 11](image)

**Figure 11.** Absolute isokinetic muscle strength of knee extensors (a) and knee flexors (b) between trained and untrained adolescents in different age groups at 60°·s⁻¹ angular velocity. The values are expressed as means ± SD.

*** U12 trained vs U12 untrained, p<0.001; $$$ U16 trained vs U16 untrained, p<0.001; NS: no significant differences.
Figure 12. Absolute isokinetic muscle strength of knee extensors (a) and knee flexors (b) between trained and untrained adolescents in different age groups at $180^\circ\cdot s^{-1}$ angular velocity. The values are expressed as means ± SD.

*** U12 trained vs U12 untrained, p<0.001; $$$ U16 trained vs U16 untrained, p<0.001; NS: no significant differences.
Figure 13. Absolute isokinetic muscle strength of knee extensors (a) and knee flexors (b) between trained and untrained adolescents in different age groups at 300° angular velocity. The values are expressed as means ± SD.

*** U12 trained vs U12 untrained, p<0.001; $$$ U16 trained vs U16 untrained, p<0.001; NS: no significant differences.

The absolute isokinetic muscle strength at 60°·s⁻¹, for the trained group, showed that there is no significant difference between 12 and 14 years old trained boys, for both knee extension
and flexion. However, significant differences were found in 16 years trained boys compared to 14 (p<0.001) and to 12 years (p<0.001) for the knee extension and the knee flexion. The changes between 14 and 16 years old trained boys for the knee extensors were 38.11% (or 62.6 Nm) and for the knee flexors 37.1% (or 34.6 Nm) (Figure 14a). At the same angular velocity for the untrained group, however, the APT values were significantly higher for the 14-year-olds, compared to 12-year-olds for knee extensors (34.64% or 37.7 Nm) and flexors (46.71% or 25.1 Nm) (p<0.01). Similarly, significant differences were observed between the 12 years old untrained boys and 16-year-olds at knee extension (p<0.01) and at knee flexion (p<0.001) (Figure 14b). In contrast, there were no significant differences in PT between the 14 and 16 years old untrained adolescents for extension and flexion (Figure 14b).
Figure 14. Changes (%) in absolute peak torque values of knee extensors and knee flexors at 60°·s⁻¹ angular velocity among different age groups for trained (a), and untrained (b) adolescents.

†† U12 untrained vs U14 untrained, p<0.01; !! U12 untrained vs U16 untrained, p<0.01; !!! U12 untrained vs U16 untrained, p<0.001; ⃝���� U12 trained vs U16 trained, p<0.001; §§§ U14 trained vs U16 trained, p<0.001; NS: no significant differences.

Similar results were found for the APT at 180°·s⁻¹, for trained adolescents. No significant differences were observed between 12 and 14 years old trained boys in extension and flexion.
Nonetheless, significantly higher absolute values were found for the 16 years old trained boys in comparison to a) the 12-year-olds (p<0.001) and b) with the 14-year-olds at knee extension (p<0.001; 32.51 % and 38.5 Nm) and knee flexion (p<0.001; 44.56% and 28.5 Nm) (Figure 15a). For the untrained group, the 14 years old untrained boys had significantly higher APT values, in comparison to the 12-year-olds, at knee extension (p<0.001; 42.42% and 31.6 Nm) and knee flexion (p<0.01; 56.47% and 20.6 Nm). Likewise, significant differences were observed between the 12 years old untrained boys and 16-year-olds, at knee extension and flexion (p<0.001) (Figure 15b). However, no significant differences were found in APT between the 14 and 16 years old untrained adolescents for extension and flexion (Figure 15b).
Figure 15. Changes (%) in absolute peak torque values of knee extensors and knee flexors at 180°·s⁻¹ angular velocity among different age groups for trained (a), and untrained (b) adolescents.

†† U12 untrained vs U14 untrained, p<0.01; ††† U12 untrained vs U14 untrained, p<0.001; !!! U12 untrained vs U16 untrained, p<0.001; ⚫⚫⚫ U12 trained vs U16 trained, p<0.001; ⚫⚫⚫⚫ U14 trained vs U16 trained, p<0.001; NS: no significant differences.
Similar patterns were observed at the high angular velocity of 300°·s⁻¹, in extension and flexion, for the trained and untrained boys. In particular, for the trained adolescents, there were no significant differences between the 12 and 14 years old trained boys in extension and flexion. On the other hand, significant differences were found in the APT values of the 16-year-olds, compared to the 14 years old trained boys, at knee extension (35.12% or 31.3 Nm) and knee flexion (30.51% or 16.7 Nm), (p<0.001); as well as compared to the 12-year-olds (p<0.001) (Figure 16a). The untrained group presented no significant differences in APT values between 14 and 16 years old untrained adolescents at extension and flexion. On the contrary, significant differences were found between the 12 and 14 years old untrained boys in extensors (51.54% or 28.8 Nm) and flexors (71.26% or 21.0 Nm) of the knee joint (p<0.001), as well as between 12 and 16-year-olds for both knee extensors and flexors (p<0.001) (Figure 16b).
Figure 16. Changes (%) in absolute peak torque values of knee extensors and knee flexors at 300°·s⁻¹ angular velocity among different age groups for trained (a), and untrained (b) adolescents.

+++ U12 untrained vs U14 untrained, p<0.001; !!! U12 untrained vs U16 untrained, p<0.001; ⬤⬤⬤ U12 trained vs U16 trained, p<0.001; §§§ U14 trained vs U16 trained, p<0.001; NS: no significant differences.
Figure 17 presents the APT values of the knee extensors and knee flexors and their quantitative differences in Nm. In addition, the percentage decrease in APT is presented, from slower to faster angular velocities, as well as the H:Q strength ratio, across angular velocities and age groups, for the trained and untrained adolescents. Generally, the isokinetic muscle strength of Q was higher than the H muscle for the trained, in comparison with the untrained adolescents, at all angular velocities. These differences exists for all age groups. In other words, the APT of Q is significantly higher compared to H at all angular velocities. The PT exerted during concentric actions is maximum at slow angular velocities and decreases with increase in angular velocity. The H:Q strength ratio, which was observed during the high angular velocity, showed that the action of H worked as fast twitch muscle fibers, which leads to the conclusion that PT was developed during the highest angular velocities.

Most commonly, the absolute muscle strength of the Q and H increases linearly with the biological age; however, some exceptions were observed. More specifically, for the knee extensors at 300°·s⁻¹, the 12 years old trained boys (Q= 94.9 Nm) had slightly higher values compared to 14 years old trained adolescents (Q= 89.3 Nm). Similarly, at 60°·s⁻¹, the untrained 14-year-olds (Q= 146.3 Nm) presented marginally higher PT values, in comparison with the 16 years old untrained adolescents (Q= 143.8 Nm). Furthermore, for the knee flexors at 180 and 300°·s⁻¹, the 12 years old trained boys showed slightly higher values (68.5 Nm and 55.5 Nm respectively) in comparison with the trained 14-year-olds (64.1 Nm and 54.8 Nm respectively) (Figure 17).

In 12 years old trained boys, at 60°·s⁻¹, the difference between PT of H (81.5 Nm) and Q (149.6 Nm) was -68 Nm and the percentage H:Q% was 54.9%. Higher H:Q% was noted in the higher angular velocities (180 and 300°·s⁻¹). In the untrained group of the same age, the PTs were
53.8 and 108.7 Nm for H and respectively. The difference in Nm between H and Q was -54.9 Nm and the H:Q% 50.6. For the angular velocity of 180°·s⁻¹, the difference in Nm between H (68.5 Nm) and Q (113.5 Nm) was -45 Nm for the trained group and -39.9 Nm, (H= 36.5 Nm and Q=76.4 Nm) for the untrained adolescents, with H:Q%= 48.3%. Likewise, at 300°·s⁻¹, the trained group presented greater difference (-39.5 Nm) in comparison with the untrained group (-26.5 Nm) between the H (55.5 Nm and 29.5 Nm respectively) and Q (94.9 Nm and 55.9 Nm respectively) with H:Q%= 53.2. The decrease % in 12 years old trained boys, between the different angular velocities, showed that at 60°·s⁻¹ - 180°·s⁻¹ was -8.2 Nm, at 180°·s⁻¹ - 300°·s⁻¹= 2.7 Nm and at 60°·s⁻¹ - 300°·s⁻¹= -4.6 Nm. The decrease % for the untrained boys was at 60°·s⁻¹ - 180°·s⁻¹= 2.4 Nm, at 180°·s⁻¹ - 300°·s⁻¹= -7.5 Nm and at 60°·s⁻¹ - 300°·s⁻¹= -3.3 Nm (Figure 17).

For the 14 years old trained boys at 60°·s⁻¹, the difference between H (93.2 Nm) and Q (164.4 Nm) was -71.2 Nm, H:Q%= 56.6% and for the untrained was -67.4 Nm (H= 78.9 Nm and Q= 146.3 Nm), H:Q%= 54.5%. The H:Q strength ratio was higher at all angular velocities for the trained boys in comparison with the untrained. At the angular velocity of 180°·s⁻¹, the difference in Nm between the H (64.1 Nm) and Q (89.3 Nm) for the trained boys was -54.4 Nm and for the untrained boys -50.9 Nm (H= 57.1 and Q= 108.1). Furthermore, at 300°·s⁻¹, the difference in Nm for the trained boys was -34.5 Nm (H= 54.8 Nm and Q= 89.3 Nm) and for the untrained was -34.3 Nm (H= 50.4 Nm and Q= 84.7 Nm). The decrease % in the 14 years old trained boys between the different angular velocities showed that at 60°·s⁻¹ - 180°·s⁻¹ was 3.3 Nm, at 180°·s⁻¹ - 300°·s⁻¹= -10.1 Nm and at 60°·s⁻¹ - 300°·s⁻¹= -4.5 Nm. The decrease % for the untrained boys at 60°·s⁻¹ - 180°·s⁻¹= 1.4 Nm, at 180°·s⁻¹ - 300°·s⁻¹= -9.9 Nm and at 60°·s⁻¹ - 300°·s⁻¹= -6.0 Nm (Figure 17).
The 16 years old trained adolescents for the 60°·s⁻¹ showed a -99.3 Nm difference between the H (127.8 Nm) and Q (227.0 Nm), (H:Q%= 56.5%) while the difference in Nm for the untrained group was -56.3 Nm (H=87.5 Nm and Q= 143.8 Nm), (H:Q%= 61.5%). For the 180°·s⁻¹, the difference in Nm was -64.4 Nm (H=92.6 Nm and Q= 157.0 Nm) for the trained boys and -58.3 Nm (H= 67.9 Nm and Q= 126.2 Nm) for the untrained. The H:Q strength ratio was 59.6% for the trained boys and 54.9% for the untrained. Moreover, at 300°·s⁻¹, the difference in Nm for the trained adolescents between the H (71.5 Nm) and Q (120.6 Nm) was -49.1 Nm, (H:Q%= 59.5%); and for the untrained -35.2 Nm (H= 53.1 Nm and Q= 88.4 Nm), H:Q%= 61.2%. The decrease % between the different angular velocities in the 16 years old trained adolescents for 60°·s⁻¹ - 180°·s⁻¹, was 3.3 Nm, at 180°·s⁻¹ - 300°·s⁻¹= -0.3 Nm and at 60°·s⁻¹ - 300°·s⁻¹= -2.8 Nm. The decrease % for the untrained boys of the same age was, at 60°·s⁻¹ - 180°·s⁻¹= 10.2 Nm, at 180°·s⁻¹ - 300°·s⁻¹= -8.2 Nm and at 60°·s⁻¹ - 300°·s⁻¹= 0.7 Nm (Figure 17).

<table>
<thead>
<tr>
<th>U12 (Trained)</th>
<th>Decrease %</th>
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<tr>
<td>Quadriceps</td>
<td>149.6 113.5 94.9</td>
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<td>H:Q (%)</td>
<td>54.9 59.8 59.2</td>
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Figure 17. Absolute peak torque values of the knee extensors and knee flexors, their quantitative differences expressed in Nm, their percent decrease from slower to faster angular velocity and the H:Q strength ratio across angular velocities and age groups for trained and untrained adolescents (mean ± SD).
5. Discussion

The present study examined two major sections that are the most important factors in physical performance. These are the cardiorespiratory and neuromuscular function (strength) of Q and H, between trained young soccer players and untrained young boys with the same biological age (12, 14 and 16 years). The research hypotheses for the current study were confirmed and the outcomes verify that \( \dot{V}O_2 \), both in absolute and relative values, was higher in the trained group than in the untrained group. Such elevation of the \( \dot{V}O_2_{\text{max}} \) in the trained group was likely caused by a) an increase in metabolic capacity, b) low running economy or c) the combination of previous training and hereditary endowment. The significant difference that was found in height between the 12 years old trained and untrained adolescents, was not due to adjustments from training, but maybe due to the fact that in soccer, as in all sports, the young athletes constitute a highly select group chosen on the basis of size and technical skills.

Maximal oxygen uptake (\( \dot{V}O_2_{\text{max}} \)) in adults

Several studies have determined the \( \dot{V}O_2_{\text{max}} \) for male elite players and reported mean values ranging from 50 to 75 ml·kg\(^{-1}\)·min\(^{-1}\) (Reilly, 2003; Davis et al., 1992; Stølen et al., 2005; Nowacki et al., 1988; Verstappen and Bovens, 1989; White et al., 1988). Generally, the values of \( \dot{V}O_2_{\text{max}} \) in soccer players are similar to those obtained in other team sports, but are considerable lower than values from elite athletes within endurance sports, where \( \dot{V}O_2_{\text{max}} \) levels are higher than 80 ml·kg\(^{-1}\)·min\(^{-1}\) (Reilly et al., 1990). The higher values that have been reported by tests on the treadmill were from a German club with \( \dot{V}O_2_{\text{max}} \) values of 69.2 (±7.8) ml·kg\(^{-1}\)·min\(^{-1}\) (Nowacki et al., 1988). Values for professional soccer players tend to be higher than amateurs, as
is the level of competition. Ekblom (1986) studied Swedish recreational players and cited values from 45 to 50 ml·kg\(^{-1}\)·min\(^{-1}\).

\(\dot{V}O_2\)\(_{\text{max}}\) in youth

\(\dot{V}O_2\)\(_{\text{max}}\) in youth has not been extensively studied in comparison with adults. At the present study, the \(\dot{V}O_2\)\(_{\text{max}}\) for the 12 years old soccer players was 57 ± 5.9 ml·kg\(^{-1}\)·min\(^{-1}\), while other researchers reported values ranging from 50 to 56 ml·kg\(^{-1}\)·min\(^{-1}\) (Berg et al., 1985; Bell, 1988). Additionally, the \(\dot{V}O_2\)\(_{\text{max}}\) for the 14 years old trained boys was 65.1 ±12.8 ml·kg\(^{-1}\)·min\(^{-1}\), slightly higher compared to the results reported by other studies, which ranged from 50 to 59 ml·kg\(^{-1}\)·min\(^{-1}\) (Jones and Helms, 1993). Moreover, the \(\dot{V}O_2\)\(_{\text{max}}\) for the 16 years old soccer players was 57.9 ± 7.1 ml·kg\(^{-1}\)·min\(^{-1}\), which is in agreement with the results reported by previous studies, ranging from 55.7 to 60.2 ml·kg\(^{-1}\)·min\(^{-1}\) (Jankovic et al., 1993; Jones and Helms, 1993; Hugg, 1996; Franks et al., 1999). The results of the current study found significantly higher absolute and relative values in \(\dot{V}O_2\)\(_{\text{max}}\) for the trained compared to untrained boys in all ages. This is in agreement with Vamvakoudis et al. (2007) who found that \(\dot{V}O_2\)\(_{\text{max}}\) was higher in 12 and 13 years old trained boys, in comparison with the untrained. Furthermore, Williams and Reilly (2000) found that training significantly increased \(\dot{V}O_2\)\(_{\text{max}}\) in prepubertal boys. They pointed out that the higher values of \(\dot{V}O_2\)\(_{\text{max}}\) of the trained adolescents, compared to the untrained were due to the large stroke volume (SV). Similarly, Matos and Winsley (2007), in a cross-sectional study of trained and untrained children suggested that cardiac size and function, thus ultimately stroke volume (SV), are greater in trained children. On the other hand, Strøyer et al. (2004) did not find differences in \(\dot{V}O_2\)\(_{\text{max}}\) between the two youngest groups (elite / non elite), either in absolute or in relative values. However, they did not examine the biological age of the adolescents. A few
studies of young soccer players who took the biological age into consideration found varying results. Baxter – Jones et al. (1995) as well as Cacciari et al. (1990) recorded no significant difference between trained and controls in prepubertal boys (10 years old). Conversely, Metaxas et al. (2014) found that $\dot{V}O_{2\text{max}}$, expressed in ml·min$^{-1}$, was significantly higher in 13 years old soccer players in comparison with the 11 year-olds. They concluded that the absolute $\dot{V}O_{2\text{max}}$ is highly related with age. However, the relative $\dot{V}O_{2\text{max}}$ (ml·kg$^{-1}$·min$^{-1}$) did not significantly differ between the ages examined (11, 13, 15 years). Nevertheless, the results of the present study showed that there were significant differences between the 12 and 14 years old soccer players both in absolute and relative $\dot{V}O_{2\text{max}}$ values ($p<0.01$). In addition, significant differences were observed between the 14 and 16 years old trained boys, only in the relative $\dot{V}O_{2\text{max}}$ values. The lower HR$_{\text{rest}}$ and BP$_{\text{rest}}$ values that were found in the trained group, compared to the untrained, is a phenomenon due to the adjustments from training. While training is a stimulus for increased GH and T levels at the biological ages of 13 and 16 years, it has no effects in prepuberty (10 years) (Zakas et al., 1994). The difference in HR$_{\text{max}}$ that was found between the trained and untrained boys at the age group of 16 year-olds may be due to a) genetic factors, or b) the fact that the untrained could not continue to exercise during maximal workload for a long time period, because of rapid fatigue. It must be noted that the HR$_{\text{max}}$ do not change with and by training.

**Concentric isokinetic muscle strength**

As noted earlier, soccer is a complicated sport involving a myriad set of activities such as jumps, feints and directional speed changes. The events put pressure on the lower limbs, showing the need for the development of muscle strength in soccer players. For this purpose, maximal
muscular strength is a fundamental quality that influences power performance (Hoff and Helgerud, 2004). To be specific, the H strength is crucial in soccer players for joint stabilization during tasks and especially in eccentric actions (Cometti et al., 2001). When the muscle strength of a pre-pubertal is examined on the isokinetic dynamometer, it is necessary to emphasize whether the muscle strength will be expressed in absolute values, as the examinee is seated and the body weight is isolated; or in relative values, considering the body mass and height of the examinee. It should be also clarified if the values of H:Q strength ratios are expressed from the conventional or from the functional (eccentric-concentric) H:Q ratio. It must be pointed out that during eccentric contraction, more force is produced, therefore the “functional” or mixed ratio (H eccentric: Q concentric) is higher.

Generally, the torque – velocity relationship in young populations indicates a similar, adult-like pattern, namely as angular velocity increased PT decreased. In this study, Q muscle strength was greater than H at all angular velocities and in all ages. The greatest difference in PT, between Q and H muscle groups, is shown at a slow angular velocity (i.e. 60°·s\(^{-1}\)). As the angular velocity increased, the differences in strength (Nm) between Q and H decreased. At slow angular velocity (i.e. 60°·s\(^{-1}\)) PT is shown in the beginning of the ROM, while at the fast angular velocity (i.e. 300°·s\(^{-1}\)), PT is shown towards the end of the movement of the knee joint.

The results obtained in this study showed that the absolute and the relative (Appendix 1) isokinetic muscle strength of knee extensors and knee flexors were significantly higher in the 12 and 16 years old trained boys, in comparison with the untrained of the same age at all angular velocities. However, it is noteworthy that there were no significant differences in the APT values of the 14 year-olds group, between trained and untrained, for both Q and H at all angular velocities. Our results are in agreement with other studies for young soccer players (Capranica et
al., 1992) who have documented no difference in isokinetic strength of the lower limbs in prepubertal trained boys, compared to non-athletes and in disagreement with all previous studies that have investigated the Q and H strength in isokinetic dynamometer, between groups with a varying CA. Nevertheless, significant differences were observed in the RPT values for that group between the trained and untrained adolescents at all angular velocities; with an exception of the fast angular velocity of 300°·s⁻¹ for the knee extensors and knee flexors (Appendix 1, 8). That finding can be explained by the fact that boys in this age are insufficient of developing maximum torque in relation to time (Mandroukas & Heller, 2019). Blimkie et al. (1989) put across the idea that a complex collaboration of different aspects might lead to the development of isokinetic leg strength during growth and maturation. Further, different cross-sectional studies have shown a significant association between stature, body mass and isokinetic leg strength (De Ste Croix et al., 1999; Housh et al., 1995). Some studies showed an increase in muscle strength of Q and H in untrained children during growth and developments (Kanehisa et al., 1995; Pääsuke et al., 2001). Boys in biological age of 14 years are in a transition period to adolescent (adulthood) that is often viewed within the context of sexual maturation and stature growth. The biological events that occur are complex and include changes to the nervous and endocrine system (Beunen & Malina, 1988).

The results of the current study clearly showed that there is a positive increase in H and Q absolute muscle strength with the increase of age across all measured angular velocities. It should be noted that this positive increase occurs despite the increase in body weight and height. In all angular velocities (60, 180, 300°·s⁻¹) the absolute muscle strength for the Q muscle in the 12 year-olds group has almost twice the strength of the H muscles (the ipsilateral H muscles). With increasing age, this difference between Q and H in muscle strength slightly decreases. The
absolute muscle strength of Q and H increases linearly with biological age. The H:Q strength ratio changes with angular velocity. As angular velocity increases, the strength percent increase is greater in the H muscle than the Q muscle. The Q is a pennate muscle, meaning all of its muscle fibers are attached to a central tendon and it contracts on a linear axis. The fibers of the Q muscle have a greater pennation angle, which translates that this muscle group produces higher strength but implies lower speed. When the knee is extended from a seated position on the dynamometer, the Q muscle is in a favorable position. Conversely, the contraction of H from the same position, places the knee in a disadvantageous state of rapid flexion. The question that arises is whether the greater strength of Q, compared to H muscles, can only be attributed to Q’s greater muscle mass; or whether it can also be attributed to the position of the body at the measuring point, where the Q muscles, due to functional anatomical position is able to make advantageous biomechanics contractions; or even whether the soccer training of the H muscle is inadequate, perhaps it is unwittingly overlooked in practice and so is not exercised to the same extent as the Q muscles.

It is well known that there is a significant difference in muscle strength between children and adults, even when it is normalized to body size. Regarding maximum effort, either for the determination of \( \dot{V}O_2_{\text{max}} \) or muscle strength, the children recruit a smaller percentage of their motor-unit pool than adults (Grosset et al., 2008). The untrained young boys in everyday life, as well as in schools’ Physical Education, are deprived from maximum physical activity, resulting in reduced coordination and rapid fatigue.

All the components for the increase of muscle strength and \( \dot{V}O_2_{\text{max}} \) may be due to neural adaptations and metabolic capacity. More specifically, Behm and Sale (1993) described a number of factors that characterized the trained adolescents, such as selective activation of
muscles, synchronization, increased reflex potential, increased recruitment of motor units and increased co-contractions of antagonists.

**Muscle strength and Joint ROM**

The joint ROM is an important factor in human performance (Mandroukas et al., 2014). Despite the fact that the etiology of muscle tightness remains obscure, hard physical exercise over a long period and exercise within a restricted range seem to have a cumulative unfavorable effect on ROM (Ekstrand and Gillquist, 1983a, b). Tightness of the Q muscle (< 52° knee flexion) had an increased risk of H injury (Gabbe et al., 2005). Although there is no scientific evidence that ROM is a means of preventing sports injuries, experimental feedback of coaches and athletes suggests that increased muscle strength is associated with shortening of the muscle. The relationship of function between H and Q, in terms of the strength and length of muscle balance plays a key role in the movement of the knee joint, which is why it is of great interest and it requires special attention in sport medicine and rehabilitation. The tightness of these two muscles (H and Q), has a direct influence on the position of the lumbar spine. All the above have a negative impact on the performance of the athlete, because they cause uncoordinated movement patterns and technical skills errors. The high frequency of sports injuries related to H and Q muscle groups in soccer has drawn researchers’ attention in the field of sports medicine and rehabilitation. The disproportionally greater increase in Q muscle strength, compared to H muscle strength, and the lack of proper flexibility programs for improving joint ROM, result in imbalance of the knee joint. As a consequence, the strong but short Q muscle and the weaker and inflexible H muscle are both more susceptible to injury.
The present study examined two postural muscles, Q and H, which tend to tighten by hard and monotonous training. The shortening of resting length in H and Q muscles may not be the crucial factor for a potential decrease in strength when assessed in the isokinetic dynamometer, because the PT is not achieved at the endpoint during joint extension. On the contrary, the shortening of the muscles may constitute them more prone to injuries, when the knee joint assumes an overextended position during practice or a soccer game. The strength assessment of these muscles is usually performed on an isokinetic dynamometer during an open kinetic chain movement. There are no ground reaction forces on the foot and shank, but only on the thigh and trunk. However, because the isokinetic dynamometer does not generate a natural movement, the values of the H:Q strength ratio, despite the useful information they provide, must be interpreted carefully. If, for example, some H:Q ratios approach 0.60, they may be used as a key factor in preventing sports injuries, because there are multiple factors that contribute to an injury in the knee joint, which cannot be easily measured and evaluated. In practice, the H:Q strength ratio is tested at completely different joint angles with different speeds, using the weight of the body, which may possibly increase the burden on the knee joint approximately tenfold, over that exerted on the joint by the isokinetic dynamometer. In soccer player movements, all leg joints and muscle groups are participating. They are closed-kinetic-chain movements that require the body to work synergistically with the muscles and joints in an integrated and coordinated fashion (Newman et al., 2004). A critical view of this study’s results on the relation of anterior and posterior thigh muscle strength, by no means challenges the widely accepted view that when these muscles have adequate and balanced strength, they stabilize joints, hence protecting from injury. The data on H:Q strength ratio produced in the present study may be useful but, at least at young ages, it is doubtful whether this data can provide a definitive answer on how to develop a
tool for preventing H or Q muscle injuries. With respect to adult soccer players, whose demands of muscle strength and endurance for maximum performance are great, a high H:Q strength ratio is necessary and beneficial and may be able to protect the knee joint from excessive and burdensome loads and have preventive effect. It has been suggested that a disproportionally small H:Q strength ratio may be related to risk of lower extremity injuries (Ahmad et al., 2006; Grygorowicz et al., 2010; O'Sullivan and Burns, 2009).

The present study examined the muscle strength of the anterior and posterior thigh muscle. It was not within the scope of this study to determine whether isokinetic strength and H:Q ratio were predictive of injury risk or the possible mechanisms involved in the injuries. However, it must be pointed out that the relationship of function between H and Q muscles in terms of strength and muscle balance in practice, plays a key role in the movement of the knee joint, which is why it is of great interest and given special attention, both in sport medicine, as well as in the rehabilitation settings. Soccer players need both in order to perform high speed running and carry out exercises with a wide range of hip flexion and knee strength, such as stretching the H muscles for optimal performance.

From the analysis of the training performed by the soccer players who participated in this study, it was observed that the H muscles are not routinely stimulated. The H muscle group received no specific training with eccentric exercise. In movements with additional weights, players mostly follow a flexion – extension exercise of the knee, without particular emphasis on training H muscles. It should be noted that the Q muscle workout is easier to perform, in comparison with the H muscle. This has no relationship with the amount of training, but with the positions and starting points, from which the training session is performed. Focused exertion of Q muscles can easily be performed from a seated position, as well as from many alternative
positions, whereas the corresponding training of the H muscle is more complex and depends, not only on the position of the isokinetic dynamometer, but also on a starting position, limited to that which places the maximum burden on the muscle group in question. Despite the emphasis during training, which stresses the fact that knee flexor strength in extremely important in soccer players for joint stabilization during various tasks, it seems that strength training of H muscles remains inadequate.
Limitation of the present study

One of the limitations of the present study is that due to the scope of this investigation, the ROM of H and Q muscles was not measured. It is known that an imbalance in the relationship of ROM between the H and Q muscles is crucial for the movement of the knee joint. Nevertheless, this study considers the absolute and relative muscle strength of H and Q muscles, as well as the H:Q strength ratio, which is beneficial for the protection of the knee joint and for preventing sport injuries. In addition, the sample size who participated in the study is below the recommendations by Hopkins (2000), as most studies involving children. However, there is great confidence in the results, since, in contrast to other studies, the biological age during the selection of the sample was considered. This has reduced any potential deviations, caused due to different maturity levels of children and eliminated any outliers. Furthermore, the participants of both groups followed the same training process, described in the methods section, which also enhances the accuracy of the findings.
6. Conclusions

The results of the present study can be used as normative data for young soccer players, as well as for the general population of young boys, throughout the developmental ages (12, 14, 16) with regards to the cardiorespiratory system and to the H and Q muscle strength. This knowledge can be used as a springboard to more efficient strengthening of the involved muscles. The findings of the present study will be a useful tool for training purposes and will provide important information for more effective training programs, accompanied by additional exercises on the field.

It is noted that, regardless of the high reliability of the isokinetic dynamometer in assessing muscle strength, this should not become the only criterion for evaluating the progress of the soccer player, who is under rehabilitation treatment. The improvement of muscle strength and functionality of the knee extensors and knee flexors should also include side cutting maneuver, neuromuscular and flexibility training of the muscles in question. Strength testing can be used to identify gross muscle weakness or functional imbalances between legs and between agonist and antagonist muscle groups of the same leg, such as knee flexor and knee extensor muscle groups.

This study assessed children of developmental ages of 12, 14 and 16 years old. It has to be noted, that the results of the laboratory measurements will have to be accompanied by practical tests on the field. Additionally, the laboratory measurements, as well as the tests on the field, show certain peculiarities. It is often made clear, that children are not a proportional diminution of adults, but a completely separate category. This means that extensive attention needs to be applied to the methodology followed during the assessment. The trained young
individuals, who are familiar with physical exercise, are responding satisfactorily, in regards to the requirements and the scope of this exercise. The difficulties in maximum performance are emerging in younger children and more evidently in the untrained ones. In this study, for the determination of $\dot{V}O_2_{\text{max}}$, the treadmill was used, as it is a realistic way of running for children. At the same time, this apparatus offers the possibility of altering the inclination (grade) for maximum loading of the central cardiovascular system. In the treadmill however, great balance and coordination of movement is needed, elements with which, the untrained need familiarisation, motivation and stimulation, with many repetitions. Despite the cost, the tests in the laboratory provide results of high accuracy. Deciphering those results correctly and providing tailored advice and training plans, can offer a great incentive for the maintenance and improvement of the physical condition and overall well-being of any individual.
References


Zakas, A., Mandroukas, K., Karamouzis, G., & Panagiotopoulou, G. (1994). Physical training, growth hormone and testosterone levels and blood pressure in prepubertal, pubertal and


Appendices

Appendix 1

RELATIVE VALUES
ISOKINETIC MEASUREMENTS FOR KNEE FLEXORS AND EXTENSORS

Relative isokinetic knee extension and flexion (60°·s⁻¹)

(a) 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
Knee extension 60°·s⁻¹ (Torque Nm)

(b) 2.5 2.0 1.5 1.0 0.5 0.0
Knee flexion 60°·s⁻¹ (Torque Nm)
Figure. Relative isokinetic muscle strength of knee extensors (a) and knee flexors (b) between trained and untrained subjects in different age groups at 60°·s⁻¹ angular velocity. The values are expressed as means ± SD.

*** U12 trained vs U12 untrained, p<0.001; ## U14 trained vs U14 untrained, p<0.01; ### U14 trained vs U14 untrained, p<0.001; $$$ U16 trained vs U16 untrained, p<0.001; NS: no significant differences.
Figure. Changes (%) in relative peak torque values of knee extensors and knee flexors at 60°·s⁻¹ angular velocity among different age groups for trained (a), and untrained (b) subjects.

† U12 untrained vs U14 untrained, p<0.05; †† U12 untrained vs U14 untrained, p<0.01; †† † U12 untrained vs U16 untrained, p<0.01; † U14 untrained vs U16 untrained, p<0.05; ⊙⊙⊙ U12 trained vs U16 trained, p<0.001; §§ U14 trained vs U16 trained, p<0.01; §§§ U14 trained vs U16 trained, p<0.001; NS: no significant differences.

Relative isokinetic knee extension and flexion (180°·s⁻¹)
Figure. Relative isokinetic muscle strength of knee extensors (a) and knee flexors (b) between trained and untrained subjects in different age groups at 180°·s⁻¹ angular velocity. The values are expressed as means ± SD.

*** U12 trained vs U12 untrained, p<0.001; # U14 trained vs U14 untrained, p<0.05; $$$ U16 trained vs U16 untrained, p<0.001.
Figure. Changes (%) in relative peak torque values of knee extensors and knee flexors at 180°·s⁻¹ angular velocity among different age groups for trained (a), and untrained (b) subjects.

†† U12 untrained vs U14 untrained, p<0.01; !!! U12 untrained vs U16 untrained, p<0.001; ¥ U12 trained vs U14 trained, p<0.05; §§§ U14 trained vs U16 trained, p<0.001; § U14 trained vs U16 trained, p<0.05; NS: no significant differences.

**Relative isokinetic knee extension and flexion (300°·s⁻¹)**

![Graph showing relative isokinetic knee extension and flexion for trained and untrained subjects at 300°·s⁻¹.](image)
Figure. Relative isokinetic muscle strength of knee extensors (a) and knee flexors (b) between trained and untrained subjects in different age groups at $300^\circ \cdot s^{-1}$ angular velocity. The values are expressed as means ± SD.

*** U12 trained vs U12 untrained, $p<0.001$; # U14 trained vs U14 untrained, $p<0.05$; $$$ U16 trained vs U16 untrained, $p<0.001$; NS: no significant differences.
Figure. Changes (%) in relative peak torque values of knee extensors and knee flexors at 300°·s⁻¹ angular velocity among different age groups for trained (a), and untrained (b) subjects.

††† U12 untrained vs U14 untrained, p<0.001; †† U12 untrained vs U16 untrained, p<0.01; † U12 untrained vs U16 untrained, p<0.01; ¥¥ U14 trained vs U16 trained, p<0.01; §§ U14 trained vs U16 trained, p<0.01; NS: no significant differences.
Appendix 2

Letter of invitation

Dear Sir/Madam/student,

I am a PhD student at Charles University in Prague. I would like to invite you to consider taking part in a voluntary research that I will strongly undertake. The research aim of the project is to examine the differences in aerobic power and isokinetic muscle strength between young soccer players and untrained boys of the same age. This will require 1 to 2 visits to the Laboratory of Exercise Physiology and Ergometry and will involve isokinetic contractions of knee flexors and extensors on an isokinetic dynamometer as well as a maximal exercise test for determination of oxygen uptake on a motorised treadmill. Before you decide whether you would like to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, parents and/or relatives if you wish. Ask me if there is anything that is not clear, or if you would like more information (contact number and email is at the end of this information sheet). Please take your time to decide whether or not you wish to take part. However, if you do not want to take part then you will not receive any more information regarding this study.

Regards,

Athanasios Mandroukas (Researcher)

Name:……………………………………………………………………………………………………………………………
Address:…………………………………………………………………………………………………………………………
Contact Tel No:……………………………………………………………………………………………………………………

I would like to take part in this study (tick box if agree) ☐
I would like to ask some questions before I decide (tick box if agree) ☐
I do not wish to take part (tick box if agree) ☐

Please return to
Athanasios Mandroukas
Information for Participants

Title of the study: Physiological and neuromuscular changes between young soccer players and untrained young subjects.

Background and aim of the study

Modern soccer incorporates periods of high intensity exercise interspersed with periods of lower intensity exercise. The physiological demands of soccer require players to be competent in several aspects of fitness, which include aerobic and anaerobic power, muscle strength, flexibility and agility (Ekblom, 1986; Reilly and Doran, 2003; Reilly and Thomas, 1976). During a game, soccer players perform a wide range of different exercises (e.g. rapid turns, accelerations, tackles, sidesteps and game-specific technical skills), (Bangsbo, 1994; Ekblom, 1986; Reilly and Thomas, 1976; Tumilty, 1993) and the intensity can alternate at any time. The effect that physical activity might have on the development of maximal aerobic power and muscle strength, during childhood and adolescence has been a matter of long-lasting controversy (Vamvakoudis et al., 2007; Degache et al., 2010; Gravina et al., 2008). Little information is available about the influence of the soccer training on muscular strength and aerobic power during the developmental ages. Muscle strength of the knee extensor and flexor muscles increases with age (Barber-Westin et al., 2006). Nevertheless, the strength ratio of Hamstrings (H) and quadriceps, (H:Q strength ratio), is different among ages and athletes. However, no study has attempted to investigate this relationship in young soccer players and in untrained young subjects of the same age. For these reasons is very essential to make a thorough investigation in order to examine and compare the physiological characteristics of young soccer players with a control group of young subjects with the same age, that their main physical activity will be the physical education at school. Therefore, the aim of this study is to examine the differences in aerobic power and isokinetic muscle strength between young soccer players and untrained boys of the same age.

Am I a suitable participant for the study?

We are recruiting healthy youth soccer players and untrained young boys (12, 14, 16 years old). This study includes 1 to 2 visits where you will be tested in the same protocol, and procedure. Anyone can participate in the study, unless you had a previous injury to the lower extremities in the last year. You should follow the same lifestyle program, without changing and/or remain at the same physical activity level.

Do I have to take part?

It is your decision whether or not to take part. If you decide to take part, you and your parent will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving reason. If you decide to withdraw from the study before participating in the sessions of data collection, your data may be included up to that time point of the study.
What will happen if I take part?

The assessments will take place in the Laboratory of Exercise Physiology and Ergometry.

Table 1 outlines what will happen. After looking at this, if you are keen to participate then please read the information on page 3 and 4.

<table>
<thead>
<tr>
<th>Visit number</th>
<th>Date of visit</th>
<th>Purpose of Visit</th>
<th>Duration of visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Screening

Before testing and once you have given consent, you will be screened by the researcher by use of a questionnaire. You will be asked to disclose lifestyle information (e.g. physical activity), previous injuries plus family history or medical conditions. You will also have your height and weight measured, following by warm up and stretching of your hamstring and quadriceps muscles.

Experimental design

Maximal concentric isokinetic strength of the knee extensor and flexor muscles will be measured using an isokinetic dynamometer (Cybex NORM, Humac, CA, USA) which allows recording of instantaneous isokinetic torque. Subjects will be positioned on an adjustable chair and adjusted to the apparatus with straps across the trunk, the hip and thigh. The alignment between the dynamometer rotational axis and the knee joint rotation axis (lateral femoral epicondyle) will be checked at the beginning of each trial. Range of motion will be set at 0° - 90° (0° corresponding to knee fully extended). Before each test the gravity compensation procedure will be performed according to the manufacturer’s instructions. The subjects will be directed to push as hard as possible against a shin pad secured to the distal tibia. The supporting pad will be placed
approximately 5cm closer to the lateral side of the ankle. The participants will be given standardized (verbal) encouragement by the investigator and will be asked to position their arms across the chest with each palm to the opposite shoulder during the maximal effort trials. On-line visual feedback of the instantaneous dynamometer torque will be projected on a computer screen for participants’ motivation. Subjects will warm-up by performing submaximal concentric contractions of the knee flexors and extensors for a range of speeds. The main test will involve several submaximal and maximal repetitions over a range of speeds. Passive recovery will follow between all series of measurement and for every angular velocity. From all the trials, peak and average torque will calculate for isokinetic and measurements as Hardling (1988) reported to be the best way to approximate the subjects’ real results. Passive recovery incorporating stretching will follow between all series of measurement and for every angular velocity.

**What do I have to do?**

You should maintain your usual physical activity and body weight over the duration of the intervention. Also, do not perform maximal training 24 hours before each training session. This will help to understand the reliability element of the measurements.

**What are the possible benefits of taking part in this study?**

The study will be carried for research purposes and to advance understanding about the influence of soccer training in muscle strength and aerobic power during the developmental ages as well as in which age there is the highest adaptation of soccer training. You will benefit from participating in the study, by understanding in details about the types of muscular contractions. Additionally, if there are any asymmetries in muscle strength; and the strength ratio between the knee flexors and knee extensors. Also, the results will provide you with a profile of the maximum strength of your knee flexors and extensors and about your maximal aerobic power.

**What are the possible risks of taking part in the study?**

Maximal isokinetic strength testing is a safe procedure but it should be avoided by people with cardiovascular disease. Also, during maximal aerobic power there is some risk of musculoskeletal injury. You must take some responsibility for your own safety as you are only able to take part if you have not a previous injury in their lower extremities the last year. The requirements presented previously are there to protect you and minimise risks.
To further reduce the chances of any injuries a thorough warm up will be performed prior to session. All tests will be performed by a trained operator and a first aider will be present in the building during each session. You will have to complete a medical questionnaire, and a questionnaire about your physical activity; these will highlight your capabilities and pre-screen you for any potential causes (e.g. cardiovascular) for concern. In addition, passive recovery and stretching will be implemented between all series of measurement.

**What happens if something goes wrong?**

All the experimental procedures that will be used in this study have been severely tested to ensure that they meet health and safety standards. These tests are all routinely and regularly performed on patients and healthy volunteers. The researcher who will perform the tests is trained and skilled to do so. Furthermore, emergency boxes are available in the laboratory and emergency contacts.

**Will taking part in this study be kept confidential?**

All information collected about you will be kept strictly confidential. Any information that leaves from the Laboratory will have your name and address removed so that you cannot be recognised from it.

**Who will be working on the study?**

PhD student Athanasios Mandroukas will perform all the assessments. At all times, there will be appropriately qualified personnel present during testing sessions.

**What will happen to the results of the study?**

Once the study has been completed all data will be anonymised and stored as per current data protection laws. The results will be written up for the dissertation. Your personal details (name, DOB, contact details etc.) will be kept securely by the researcher. Results will also be made available to you (the participants) on request at any time throughout the study. Data could be stored for up to ten years before it gets destroyed.

*Thank you for considering participation in the study.*
Contact for further information:

If you require further advice about this study, at any time during participation, you may contact Athanasios Mandroukas who is organising the study.

Athanasios Mandroukas: Researcher
Tel: +30 6947928933
E-mail: thanmandrou@hotmail.com / a.mandroukas@hotmail.com
Appendix 3

Pre-Test Guidelines

You are required to try and keep the following guidelines:

1. Try to get at least 24 hours rest before the familiarization and experimental sessions. It is particularly important that you do not perform any form of maximal exercise for at least 24 hours before the experimental sessions.

2. You should maintain your usual physical activity and body weight over the duration of the intervention.

3. Your sleep pattern should be stable throughout the study. Try to get adequate sleep (6-8 hours) before experimental and test sessions.

Thank you

Athanasios Mandroukas
Appendix 4

Informed Consent Form for Participants

I, the undersigned, confirm that (please tick box as appropriate):

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>I have read and understood the information about the project, as provided in the Information Sheet.</td>
</tr>
<tr>
<td>2.</td>
<td>I have been given the opportunity to ask questions about the project and my participation.</td>
</tr>
<tr>
<td>3.</td>
<td>I voluntarily agree to participate in the project.</td>
</tr>
<tr>
<td>4.</td>
<td>I understand I can withdraw at any time without giving reasons and that I will not be penalised for withdrawing nor will I be questioned on why I have withdrawn.</td>
</tr>
<tr>
<td>5.</td>
<td>The procedures regarding confidentiality have been clearly explained (e.g. use of names, pseudonyms, anonymisation of data, etc.) to me.</td>
</tr>
<tr>
<td>6.</td>
<td>I have had the opportunity to speak to Athanasios Mandroukas (Researcher) and/or Dr Bissas Athanasios (Research Supervisor).</td>
</tr>
<tr>
<td>7.</td>
<td>The use of the data in research, publications, sharing and archiving has been explained to me.</td>
</tr>
<tr>
<td>8.</td>
<td>I understand that other researchers will have access to this data only if they agree to preserve the confidentiality of the data and if they agree to the terms I have specified in this form.</td>
</tr>
<tr>
<td>9.</td>
<td>Select only one of the following:</td>
</tr>
<tr>
<td></td>
<td>• I would like my name used and understand what I have said or written as part of this study will be used in reports, publications and other research outputs so that anything I have contributed to this project can be recognised.</td>
</tr>
<tr>
<td></td>
<td>• I do not want my name used in this project.</td>
</tr>
<tr>
<td>10.</td>
<td>I, along with the Researcher, agree to sign and date this informed consent form.</td>
</tr>
<tr>
<td>11.</td>
<td>I have answered NO to all the questions in the medical questionnaire</td>
</tr>
</tbody>
</table>

**Participant and Parent:**

<table>
<thead>
<tr>
<th>Name of Participant / Parent</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

109
Thank you for your participation!

Please complete and return this form to Athanasios Mandroukas
Appendix 5

Medical Questionnaire

Please read the following questions very carefully and answer each one honestly by deleting as applicable.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>2.</td>
<td>Has your doctor ever said that you have a heart or another serious condition and that you should not participate in maximal strength testing?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>3.</td>
<td>Do you feel pain in your chest when you do physical activity?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>4.</td>
<td>In the past month, have you had chest pain when you were not doing physical activity?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>5.</td>
<td>Has your doctor ever said that you have high blood pressure?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>6.</td>
<td>Does exercise cause you to feel dizzy or faint or do you ever lose consciousness?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>7.</td>
<td>Do you ever have nausea when you exercise?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>8.</td>
<td>Do you ever feel short of breath when you exercise or have ever suffered from asthma?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>9.</td>
<td>Do you have any bone, joint, or muscular problem(s) that could be made worse by maximal strength testing or maximal training in general?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>10.</td>
<td>Are you aware of any other medical conditions (e.g. hernia) or problems that you may have and they could worsen or lead to more substantial conditions after performing maximal strength testing?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>11.</td>
<td>Is your doctor currently prescribing medication for you?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>12.</td>
<td>Do you know of any other reason why you should not do physical activity?</td>
<td>Yes/No</td>
</tr>
</tbody>
</table>

I have read, understood and completed the questionnaire to the best of knowledge.

**Participant / Parent:**

---

Name of Participant / Parent | Signature | Date
# Appendix 6

## Physical activity Questionnaire

Please read the following questions very carefully and answer each one honestly (please tick box as appropriate):

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. What is your physical activity level?</td>
<td>Low activity, Moderate activity, High activity</td>
</tr>
<tr>
<td>2. At school I walk</td>
<td>Never, Seldom, Sometimes, Often, Always</td>
</tr>
<tr>
<td>3. At school I lift heavy loads</td>
<td>Never, Seldom, Sometimes, Often, Always</td>
</tr>
<tr>
<td>4. After school I am tired</td>
<td>Very often, Often, Sometimes, Seldom, Never</td>
</tr>
<tr>
<td>5. In comparison of others of my own age I think my activity is physically</td>
<td>Much heavier, Heavier, As heavy, Lighter, Much lighter</td>
</tr>
<tr>
<td>6. During leisure time I play sport</td>
<td>Never, Seldom, Sometimes, Often, Very often</td>
</tr>
<tr>
<td>7. What sport do you play most frequently</td>
<td>low intensity, medium intensity, high intensity</td>
</tr>
<tr>
<td>8. How many hours do you play a week?</td>
<td>&lt; 1 hour, 1-2 hours, 2-3 hours, 3-4 hours, &gt; 4 hours</td>
</tr>
</tbody>
</table>

I have read, understood and completed the questionnaire to the best of knowledge

**Participant / Parent:**

Name of Participant / Parent  |  |  |  
|-----------------------------| | |  
Signature  | | |  
Date
Pre-exercise Information

Name:  
Date:  
Date of Birth:  

Height:  
Weight:  

In accordance with the pre-test guidelines, please read the following questions very carefully and answer each one honestly by deleting as applicable.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Do you have any injuries that may be made worse by participation in maximal strength testing or maximal exercise in general?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>2.</td>
<td>Have you performed any maximal exercise in the last 24 hours?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>3.</td>
<td>Has your sleep pattern remained over the past couple of days?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>4.</td>
<td>Have you maintain your usual physical activity and body weight?</td>
<td>Yes/No</td>
</tr>
</tbody>
</table>

Thank you

Athanasios Mandroukas
Thessaloniki 30th of March 2017

To

Faculty of Physical Education and Sport

José Martiho 31, 162 52 Praha 6 – Veleslín

A significant part of the research entitled: “Physiological and neuromuscular changes between young soccer players and untrained young subjects. A comparison study” conducted by Athanasios Mandroukas, Ph.D. candidate of the Faculty of Physical Education and Sport, Charles University in Prague, was carried out during the years 2015 – 2016 in the framework of a Ph.D. project in the Laboratory of Sports Medicine of the Department of Physical Education and Sports Sciences, Aristotle University of Thessaloniki (AUTH). The study has been completed according to the guidelines of the Ethics Issues Code of the Research Committee of Aristotle University of Thessaloniki.

The Director of Sports Medicine Laboratory

Professor Asterios Deligiannis