ASSESSMENT OF VASCULAR HEALTH

IN CHILDREN WITH CEREBRAL PALSY

by

JUNSOO LEE

(Under the Direction of Christopher Modlesky)

ABSTRACT

The purpose of this dissertation was to assess the vascular health of ambulatory children with spastic cerebral palsy (CP). The first study aimed to determine whether the size and hemodynamics of the popliteal artery are compromised in children with CP. The main finding was that children with CP had smaller diameter, no difference in oscillatory shear index, and higher shear rate than typically developing children. However, the difference in diameter was more evident in children with bilateral than unilateral CP, and no longer significant after adjusting for the smaller leg muscles in children with CP, indicating that the size of the popliteal artery tracks the size of the muscle. The relevance of the elevated shear rate in children with CP requires further study. The second study aimed to determine if the diameter and hemodynamics of the popliteal artery are related to physical activity and function in children with CP. The main findings were that the smaller artery diameter and higher shear rate in children with CP were associated with lower functional capacity, assessed via the 6-minute walk test. Shear rate was also inversely related to performance on the progressive lateral step-up test, a measure of functional muscle strength. However, neither diameter nor shear rate was related to physical activity. These results suggest that the limited functional capacity of children with CP may suppress popliteal artery growth. The relevance of the relationship between shear rate and functional capacity warrants further investigation. The third study aimed to determine whether blood pressure measurements are consistent across arms in children with CP. The main finding was that although high intraclass correlations between blood pressure measurements from the more- and less-affected arms indicated excellent agreement, 4 of 14 children with bilateral CP exhibited a pathologically significant inter-arm difference (>10 mmHg). Thus, assessing blood pressure in both arms may be necessary in children with bilateral CP.

In conclusion, children with CP have disturbances in their vascular health that are related to their physical function, more evident in children with bilateral than unilateral CP, and require more attention from scientists and clinicians.

INDEX WORDS: Cerebral palsy; popliteal artery; arterial diameter; shear rate; physical activity; blood pressure.

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CHAPTER 1

INTRODUCTION

Cerebral palsy (CP) is a non-progressive motor disorder that occurs in the developing brain.[3] Although the associated brain injury or malformation does not progress with time, degenerative changes appear much earlier in the lifespan of children with CP compared to the general population due to muscle weakness, impaired motor control, and activity limitations.[4] Concurrently, compared to typical peers, an earlier onset of cardiovascular disease progression[5,6] and resultant increased cardiovascular disease-related mortality[7,8] is observed in those with CP. Cardiovascular disease is the third most common cause of death in individuals with CP (15 %),[9] with mortality risk increasing as the severity of CP increase.[7] Therefore, assessing cardiovascular risk factors in children with CP may provide an excellent window to understand the pathophysiology of cardiovascular disease in individuals with CP.

Traditional cardiovascular disease risk factors, such as high blood pressure, high low-density lipoprotein cholesterol, diabetes, smoking and secondhand smoke exposure, obesity, unhealthy diet, and physical inactivity, were recognized from the Framingham Heart Study.[10] Several systemic and scoping reviews have been conducted on traditional cardiovascular risk factors in individuals with CP.[11-13] These reviews showed that individuals with CP have higher blood pressure and higher prevalence of hypertension compared to the general population,[13] reduced levels of physical activity throughout their lifespans,[11,12] and elevated levels of low-density lipoprotein cholesterol compared to reference values.[12]

In addition to traditional cardiovascular risk factors studied for decades, non-traditional risk factors, including artery diameter, have emerged as important predictors of cardiovascular disease in the general population.[14-17] The incorporation of functional and structural arterial characteristics has the potential to improve the accuracy of cardiovascular disease predictions and improve the care for at-risk populations. Carotid artery diameter correlates with cardiovascular risk factors in adults[17] and incidence of cardiovascular disease.[18] Brachial artery diameter also correlates with cardiovascular risk factors.[19] These alterations observed in the estimated artery diameter among individuals with cardiovascular risk factors can potentially indicate impaired vasoregulation, which is commonly associated with the progression of atherosclerosis.

Shear stress plays a crucial role in maintaining vascular function, as consistent laminar shear stress protects against clotting,[20] preserves endothelial wall integrity,[21] and promotes the maintenance of vascular homeostasis.[22] Additionally, shear stress has the ability to regulate artery diameter by influencing the production of vasoactive mediators[23,24] and is a determinant of gene expression in endothelial cells.[25] In clinical settings, shear stress is typically measured by estimating whole blood viscosity and shear rate based on the blood flow velocity and internal diameter of the artery.[26] Engaging in physical activity can enhance vascular function and reduce the risk of cardiovascular diseases by inducing shear stress.[27] Despite increased cardiovascular risk,[28] shear rate is higher in those with spinal cord injury than in healthy adults,[29] which suggests that individuals with activity limitations may develop an atheroprotective hemodynamic environment to compensate for the adverse effect of inactivity on cardiovascular risk. However, no such study has been conducted in children, adolescents, or adults with CP.

Specifically in individuals with CP, significant changes in non-traditional risk factors for cardiovascular disease were observed over 4 years, while no changes in traditional risk factors were observed.[30] Considering higher levels of low-density lipoprotein cholesterol observed in individuals with CP compared to reference values,[12] it is reasonable to emphasize the significance of closely monitoring non-traditional risk factors. However, studies on arterial structure and its hemodynamics are lacking in children with CP. A comprehensive literature search revealed only 5 studies on arterial structure and function in children with CP.[30-34] Among these studies, only 3 studies reported artery diameter,[31,33,34] and no study exists on arterial hemodynamics in children with CP.

No group difference in carotid and brachial artery diameter between children with CP and controls was observed.[33,34] In contrast, the diameter of the infrarenal abdominal aorta was smaller in children with CP than in controls.[31] Consequently, it is suspected that the reduced exercise capacity resulting from activity limitations in children with CP may be responsible for the comparatively smaller infrarenal abdominal aorta diameter. Considering that the infrarenal abdominal aorta is responsible for supplying blood to the lower body, these results suggest that the use of arteries related to the blood supply to the lower limb may be more appropriate for investigating the relationship between vascular structure, physical activity, and cardiovascular risks in children with CP. This assumption is supported by the previous literature, which showed that a reduction in physical activity primarily affects the arteries perfusing the limbs exposed to the greatest reduction in activity.[35] The popliteal artery supplies the blood to the lower limb muscles, including the hamstring, gastrocnemius, soleus, and plantar muscles, and represents the primary source of blood to the leg and foot.[36] The relationship between the popliteal artery and physical activity has been demonstrated in other populations.[37,38] However, no such study was done in children, adolescents, or adults with CP. Therefore, the significance of assessing the relationship between popliteal artery diameter and its hemodynamics to activity limitations, which have been associated with the development of cardiovascular disease in children with CP, cannot be overstressed.

Blood pressure is one of the traditional measures that demonstrate cardiovascular disease risk. Early identification and intervention of high blood pressure in children is required to reduce cardiovascular disease risk in the future. However, there is still no clear answer regarding which arm blood pressure measurement is more appropriate for children with CP. In the general population, for consistency and comparison with standard tables and for the possibility of coarctation of the aorta, the right arm is preferred for repeated measurements of blood pressure.[39,40] On the other hand, in patients with hemiparesis, the nonparetic arm is preferred because the muscle tone in the paretic arm could influence blood pressure.[41] Additionally, a Modified Ashworth Scale score greater than 2 is associated with significantly higher blood pressure in patients with hemiplegia.[42] In children with CP, there is no widely accepted rule at the moment. In Noten's review about blood pressure in adults with CP, only half of the included studies measured blood pressure in the least affected or unaffected arm. Others used the left or right arm regardless of the affected side.[13] It is generally recommended to measure blood pressure in the right arm for consistency with standard reference tables. However, considering that spasticity could affect blood pressure measurements in children with CP, it may be more practical to measure blood pressure in the less-affected arm for these individuals and in the dominant arm for control subjects. This approach has been utilized in previous studies conducted in our lab despite the lack of conclusive evidence.

The purposes of this dissertation were following: 1) to determine if the diameter of the popliteal artery is compromised in children with CP relative to age-, sex-, and race-matched typically developing controls. We hypothesized that children with CP will have a smaller normalized popliteal artery diameter compared to typically developing controls regardless of normalization methods, 2) to determine if the shear rate and oscillatory shear index of the popliteal artery are higher and lower in children with CP relative to age-, sex-, and race-matched typically developing controls. We hypothesized that children with CP will show a higher shear rate and lower oscillatory shear index compared to typically developing children, 3) to determine if the diameter and hemodynamics of the popliteal artery are related to physical activity and function in children with CP. The study hypothesized that physical activity and functional tests of the lower limb would be directly related to the popliteal artery diameter and negatively related to the shear rate in children with CP, 4) to determine whether blood pressure measurement is consistent across arms in children with CP and to assess its impact on the diagnosis of elevated blood pressure or hypertension. We hypothesized that blood pressure in the more-affected arm would be higher than in the less-affected arm.

CHAPTER 2

LITERATURE REVIEW

2.1 Cerebral Palsy (CP)

In 2005, Bax proposed a definition for cerebral palsy (CP) as a group of irreversible disorders of the development of movement and posture, causing activity limitation attributed to nonprogressive disturbances occurring in the developing fetal or infant brain.[3] CP is diagnosed based on motor function and posture disorders that occur in early childhood, and it is the most common cause of motor deficiency in young children, occurring in 2 to 3 per 1000 live births in most European countries.[43]

2.1.1 Types of cerebral palsy

The Surveillance of Cerebral Palsy in Europe has classified CP into three main groups based on neurological signs due to the disorders in the cerebral motor systems, spastic, ataxic, and dyskinetic CP. Spastic CP patients have increased tone and pathological reflexes. Their increased tone in spasticity is characterized by an increased resistance, which depends on the velocity of movement.[44] Dyskinetic CP patients demonstrate involuntary, uncontrolled, recurring, and occasionally stereotyped movements. Ataxic CP patients show a loss of orderly muscular coordination so that their movements are performed with abnormal force, rhythm, and accuracy. They also show tremors and low muscle tone. Some patients even show mixed CP symptoms. In these cases, the child should be classified according to the dominant clinical feature.[45] Spastic type of CP is predominant, with more than 80% of individuals with CP have spasticity.[46] Spastic CP is categorized based on the anatomical pattern of involvement. Hemiplegia involves one-half of the body, diplegia involves primarily the lower extremities with mild upper extremity involvement, and quadriplegia involves all four limbs. The focus of this literature review will be on children with spastic CP.

2.1.2 Characteristics of Cerebral Palsy

While symptoms may evolve throughout a lifetime, CP itself is non-progressive, meaning that it does not worsen over time. Degenerative changes seen throughout the lifetime of people with CP stem from activity limitations caused by CP. Thus, management of activity limitation would be the best treatment option.[4] Activity limitation due to neural factors such as reduced motor drive, abnormal neural circuits, altered recruitment patterns, impaired reciprocal inhibition, and altered setting of muscle spindles[47] causes several musculoskeletal deficiencies in children with CP. Their muscle showed reduced cross-sectional area,[48] increased fat[49] and collagen,[50] and higher muscle spasticity[51] than their typically developing peers. This deterioration in muscle quality creates a vicious cycle of lowering muscle quality again.[47] These muscular weaknesses also alter the transmission of mechanical loading[52] and hinder the growth of bone, leading to children with CP having underdeveloped bones[53] compared to typically developing children.

2.1.3 Motor function of cerebral palsy

The ability to do tasks that involve large muscles in the torso, legs, and arms in people with CP is usually measured according to the gross motor function classification system (GMFCS).[54] This system has a five-point scale indicating the different levels of motor function deficit with emphasis on function in sitting and walking.[55] GMFCS I indicates that the child has the ability to walk indoors or outdoors and climb stairs without limitations, but they perform those at reduced speeds compared to typically developing children. GMFCS II indicates that the child can walk indoors and outdoors without assistive devices but experiences limited walking on uneven surfaces, inclines, and crowded places. The difference between GMFCS I and II is the limited ability to run and jump. GMFCS III indicates that the child requires the help of assistive mobility devices, such as a crane and crutches, to walk indoors and outdoors. They might use wheeled assistive mobility devices such as wheelchairs and knee walkers to move a long distance. GMFCS IV indicates that the child needs a wheelchair to move around. The difference between GMFCS III and IV is the limitation of independent mobility in children with CP who have GMFCS IV. GMFCS V indicates that the child completely lacks the ability for independent mobility even with the use of assistive devices. The reliability and validity of GMFCS for children with CP have been proven to be excellent.[56]

2.2 Cardiovascular Disease Risk Factors in the General Population

2.2.1 Blood pressure

Blood pressure is one of the traditional measures to demonstrate the cardiovascular disease risk. The prevalence of high blood pressure in children has increased significantly in Western countries,[57,58] and this high blood pressure persists into adolescence and increased cardiovascular disease risks in adulthood.[59-61] Early identification and intervention of high blood pressure in children is required to reduce cardiovascular disease risk in the future.

The association between blood pressure in children and overweight/obesity is demonstrated by several studies.[61-64] Falkner et al presented a positive association between blood pressure and body mass index among children aged from 2 to 19 years.[63] Juhola et al. showed that overweight/obesity in childhood could induce hypertension.[61] Gialamas et al.[62] suggested that body mass index is more positively related to blood pressure than height at 11 and 18 years. Furthermore, Imoisili et al.[64] revealed that weight management intervention in the pediatric population could decrease blood pressure and body mass index. These studies displayed that blood pressure is highly associated with overweight / obesity in children and can be improved by weight management intervention.

Blood pressure in children is also correlated with physical activity. Anderson et al.[65] showed a negative correlation between Physical activity and blood pressure in typically developing children. Mark & Janssen observed a negative dose-response relationship between physical activity and blood pressure in youth aged 8 to 17 years.[66] They found that certain amounts of physical activity are required to affect blood pressure. Although Hatfield et al.[67] showed that total activity count might be more strongly associated with blood pressure than moderate to vigorous physical activity in U.S. adolescents, several studies reached at different conclusions. Hay et al.[68] showed that the intensity of physical activity is associated with blood pressure, suggesting that vigorous types of physical activity should be encouraged to reduce cardiometabolic risk at young ages. Muller et al.[69] also found that children who engaged in more moderate to vigorous physical activity had lower systolic blood pressure. Cao et al.[70] demonstrated that high-intensity interval training could significantly decrease systolic blood pressure compared to moderate-intensity continuous training. These studies suggested that certain amounts and intensity of physical activity are required to decrease blood pressure and cardiovascular disease risk.

These associations between blood pressure, physical activity, and overweight/obesity are also observed in children with CP. Due to the limitation in mobility reducing the amount of physical activity and subsequent overweight/obesity in children with CP, they have a higher cardiovascular disease risk compared to typically developing children.[13,71,72] Ryan et al.[71] demonstrated that children with CP had a higher prevalence of elevated blood pressure and hypertension compared to typically developing children, and this higher prevalence is associated with their decreased total physical activity and increased time in sedentary activity. McPhee et al.[73] also demonstrated that blood pressure in ambulatory persons with CP depends on GMFCS level. In their study, the group who could walk without limitations had lower blood pressure than those who walked with limitations.

It is still controversial which arm blood pressure measurement is more appropriate for children with cerebral palsy. In the general population, for consistency and comparison with standard tables and for the possibility of coarctation of the aorta, the right arm is preferred for repeated measurements of blood pressure.[39,40] On the other hand, in patients with hemiparesis, the nonparetic arm is preferred because the muscle tone in the paretic arm could influence blood pressure.[41,74] Lin's team found that a Modified Ashworth Scale of equal or more than 2 can elevate blood pressure significantly in patients with hemiplegia.[42] In children with CP, there doesn't seem to be a widely accepted rule at the moment. In Noten's review[13] about blood pressure in adults with CP, only half of the included studies measured blood pressure in the least affected or unaffected arm. Others used the left or right arm regardless of the affected side. For comparison with standard reference tables, measuring blood pressure in the right arm would be ideal. However, there is a high possibility that spasticity might affect blood pressure in children with CP. Thus, if it is possible to directly compare the measured values of CP and typically developing children without relying on standard tables, it is reasonable to measure blood pressure in the less-affected arm in children with CP and the dominant arm in typically developing children.

The prevalence of hypertension in children and adolescents is increasing after an increase in the prevalence of obesity.[58,75] In the general population, Song et al.[75] reported that the global prevalence of hypertension increased from 2.42% in 6-year-olds and 4.51% in 14-year-olds to 00 to 4.32% and 7.89%, respectively, from the year 2001 to 2015. Children with CP are considered at high risk for high blood pressure because of limited exercise capacity and the resulting overweight. However, studies related to the prevalence of hypertension in children with CP are poor. To the best of my knowledge, only Ryan et al.[71] showed that the prevalence of pre- and hypertension in ambulatory children with CP are 11.6% and 10.5%, respectively. This higher prevalence of hypertension in children with CP compared to the general population continues to adulthood.[13] Although limited results, these suggest that children with CP have an increased risk of hypertension, and appropriate preventive interventions should be introduced from an early age in this vulnerable population.

2.2.2 Shear rate

The endothelial cells that line the inner wall of the artery are able to detect a pressure pulse, which is the difference between diastolic and systolic blood pressure, as well as the tangential stress exerted by the flowing blood. This tangential stress is referred to as shear stress. Shear stress is a biomechanical force influenced by factors such as blood flow, vessel geometry, and fluid viscosity. It is typically quantified through computational fluid dynamics models and expressed in dyn/cm^2.[76] In clinical research, shear stress is commonly determined using whole blood viscosity and shear rate, estimated from the measured blood flow velocity and the internal diameter of the artery.[26] If it's not available to measure blood viscosity, a value of 3.5 cP can be used as a substitute in most cases. The provided value is calculated under the assumption that blood behaves as a Newtonian fluid in large vessels. Shear rate refers to the speed at which neighboring layers of fluid move in relation to one another, usually measured in reciprocal seconds. Assuming the blood to be an ideal Newtonian fluid characterized by constant viscosity, steady and laminar flow, and housed within a straight, cylindrical, and inelastic vessel, Poiseuille’s law can effectively calculate the shear rate as follows:

$$Shear\ rate\ = \frac{8 \times Blood\ flow\ velocity}{Vessel\ diameter}$$

Shear stress plays a crucial role in determining endothelial cell function and gene expression. It effectively regulates arterial diameter by influencing endothelial cells' production of vasoactive mediators.[24,77] In normal laminar flow, shear stress is crucial for maintaining normal physiological vascular function, including anti-thrombotic, anti-inflammatory, anti-proliferative, and vascular homeostasis. However, when blood flow is disturbed and becomes non-laminar, shear stress plays a vital role in the pathogenesis of atherosclerotic plaque, especially in areas with disturbed flow conditions characterized by low or oscillatory shear stress.[78]

The biological function of blood vessels is regulated by shear stress, which plays a pivotal role in regulating both the normal functioning and dysfunction of the vessel wall through intricate molecular mechanisms. In vitro, shear stress levels of 1.0[79] to 1.5 Pa,[80] prompt gene expression that protects against atherosclerosis. In contrast, a shear stress level of 0.4 Pa induces the expression of genes associated with atherosclerosis.[80] Prolonged exposure to a physiologically relevant range of shear stress, particularly in straight sections of the artery experiencing unidirectional laminar flow, encourages the development of important physiological characteristics in the artery wall, promoting an anti-inflammatory, anti-thrombotic, anti-proliferative, and anti-oxidative function.[78]

The role of shear stress in regulating the inflammatory processes that initiate and enhance the growth of fibro-inflammatory lipid plaque is crucial. Shear stress has been found to influence vascular inflammation by modifying endothelial gene expression to a pro-atherogenic profile.[81] Studies have shown that non-laminar flow can lead to the gene expression of pro-inflammatory molecules in the vascular wall.[25,81] Additionally, low shear stress affects leukocyte adhesion to activated endothelium through increased expression of adhesion molecules such as ICAM-1 and VCAM-1.[82,83]

Endothelium responds to shear stress through various pathophysiological mechanisms, which depend on the type and magnitude of shear stress. When the vascular endothelium is exposed to shear stress within the normal range, it stimulates endothelial cells to release substances that have direct or indirect anti-thrombotic properties, such as prostacyclin,[84] nitric oxide,[85] calcium,[86] and thrombomodulin.[87]

Laminar shear stress has been found to inhibit the proliferation of endothelial and vascular smooth muscle cells.[88,89] The activation and proliferation of vascular smooth muscle cells are known to play a crucial role in the development of atherosclerosis. It has been observed that areas with the lowest mean shear stress tend to have the greatest intima-media thickness. Studies have shown a negative correlation between intima-media thickness and shear stress in coronary arteries,[90] carotid arteries,[91] and femoral arteries.[92]

Reactive oxygen species are known to enhance leukocyte adhesion molecule expression,[93] promote smooth muscle cell proliferation and migration, induce lipid oxidation, and disrupt vasomotor activity.[94] Shear stress plays a role in regulating the oxidative state of the vascular wall, partly by controlling the expression of various oxidase systems. Endothelial cells exposed to oscillatory shear stress exhibit a significant increase in NAD(P)H activity and production of reactive oxygen species, in contrast to the anti-oxidative state induced by laminar flow.[95]

Shear stress plays a crucial role in determining acutely and chronically arterial diameter. This adaptive process is essential for keeping mean wall shear stress within appropriate limits as required blood flow change.[24,77,96] Elevated shear stress levels result in increased wall thickness and vessel dilation, allowing shear stress values to return to normal. On the other hand, low shear stress causes a reduction in vessel diameter and leads to intima-media hyperplasia. Therefore, shear stresses trigger vasoregulatory mechanisms that, coupled with changes in arterial diameter, work to maintain a mean shear stress level.[97,98] Previous research, encompassing both animal[99] and human[100] studies, has indicated that the expansion of the arterial lumen in large arteries persists until the wall shear stress reaches a normalized state. This luminal expansion typically occurs after the wall shear stress surpasses a certain threshold level.[101] The flexibility of arterial diameter is evident in response to immediate changes in wall shear stress and adaptation to chronic variations. The radial artery diameter increases with sustained elevated shear stress,[102] while the carotid artery diameter decreases after a prolonged reduction in shear stress.[103] This phenomenon has also been observed in endurance-trained athletes and paraplegic patients; the femoral artery diameter is notably larger in the former, reflecting the increased blood flow this artery must accommodate in athletes compared to paraplegic patients.[104] Consequently, due to the smaller diameter, the shear rate is significantly higher in paraplegic patients compared to healthy controls, although it does not appear to be sufficiently high to trigger arterial diameter expansion.[104] Additionally, intima-media thickness is highest in the areas with the lowest mean wall shear stress. Intima media thickness and shear stress show a negative correlation in coronary arteries,[90] carotid arteries,[91] and femoral arteries.[92]

2.2.3 Popliteal artery

The popliteal artery starts from the distal end of the femoral artery at the adductor hiatus and continues to the anterior and posterior tibial arteries at the lower border of the popliteus muscle. It is located deep within the popliteal fossa, surrounded by the biceps femoris tendon and the two heads of gastrocnemius, covered by the popliteal vein, and crossed by the tibial nerve. The popliteal artery provides the blood to muscular branches, geniculate branches, and the anterior and posterior tibial arteries.[105] It covers lower limb muscles, including the hamstring, gastrocnemius, soleus, and plantar muscles, and represents the major source of blood to the leg and foot.[36]

Several studies demonstrate that Ultrasound measurement of blood flow,[106] velocity,[38] and diameter of popliteal artery[38,107] is reliable. Ultrasound is widely used to study the function and architectural properties of popliteal artery compared to the other peripheral arteries, such as femoral artery[108,109] and brachial artery,[35,110-112] and central arteries, such as aorta.[113]
The diameter of the popliteal artery increases with age and is also related to body surface area and gender, with males having larger arteries than females.[111,114,115] Popliteal arterial wall is also thickened with aging, like carotid artery, regardless of gender.[116] Flow-mediated dilation of popliteal artery decreased with age, similar to, but not identical to, the brachial artery.[117] Thijssen et al.[110] showed that the differences in vascular function response between peripheral arteries, such as brachial, femoral, and popliteal arteries could be influenced by artery size. Jadidi et al.[109]

also demonstrated that the distinct physiological characteristic of popliteal artery is derived from the differences in diameter size between peripheral arteries. Although popliteal artery is considered a peripheral muscular artery, the characteristics of the popliteal arterial wall are similar to central elastic arteries such as aorta, increasing in diameter and decreasing in distensibility with aging and being affected by gender, whereas arterial walls of peripheral muscular artery such as brachial and femora artery are generally less affected by aging. These characteristics might contribute to its susceptibility to aneurysm development and peripheral vascular disease.[109,113] However, aneurysm of the popliteal artery is now rare due to the reduced frequency of the horse riding and the wearing of high riding boots.[105]

A sedentary lifestyle, lack of physical activity, or immobilization could induce alteration in the function and structure of popliteal artery and promote the development of cardiovascular disease. Compliance and diameter of popliteal artery were reduced with 12 days of immobilization, and blood flow and shear rate of popliteal artery were significantly reduced during the 3 hours of sitting but increased after 12 days of immobilization mainly due to reduced compliance and diameter. Flow-mediated dilation was also decreased during the 3 hours of sitting and following 5 days of reduced physical activity but increased after 12 days of immobilization.[35,108,118] These alterations of popliteal artery properties due to immobilization are consistent with the alteration of popliteal artery properties in spinal cord injury patients. In SCI patients, the diameter was smaller, and velocity and shear rate were greater compared to healthy controls.[29] These changes are favorable to an atheroprotective environment and expected to occur in children with CP also but have not been studied yet to our knowledge. Interestingly, the degree of hypertension also affects the diameter of popliteal artery. Pinto et al.[119] showed that the diameter of popliteal artery was significantly lower in severe hypertensive patients than in moderate and mild hypertensive patients. It suggested that the higher blood pressure in children with CP could be one of the reasons for the smaller diameter of popliteal artery in children with CP compared to typically developing children.

Contrary to immobilization, exercise could induce positive arterial adaptation. Karagounis et al.[120] showed that the diameter and blood flow velocity of popliteal artery were increased in elite Judo athletes compared to controls. The wall thickness of popliteal artery decreased after 24 weeks of training.[111] Endothelial dysfunction due to 3 hours of sitting was prevented in the endurance training group.[118] 8 weeks of low volume high-intensity interval training increased the diameter of popliteal artery and decreased the shear rate.[121] Not only long-term exercise, but also short-term exercise has an impact. An acute bout of intense cycling exercise could increase the diameter and blood flow velocity of popliteal artery,[112] and small amounts of leg movement, such as fidgeting, can prevent endothelial dysfunction due to prolonged sitting.[122] These studies suggested that the function and structure of popliteal artery can be improved by exercise.

Interventions such as whole-body vibration and electromyostimulation as well can improve the function and structure of popliteal artery. Menendez et al.[123-125] performed several studies to analyze the effect of whole-body vibration and electromyostimulation on popliteal artery and showed that those interventions could increase blood flow velocity and diameter of popliteal artery in SCI patients and healthy controls. Szopa et al.[126] also demonstrated that whole-body vibration training can increase the blood flow velocity of the popliteal artery in patients with myelomeningocele. Based on the results of these studies, the effect of whole-body vibration on the popliteal artery in children with CP can be expected.

2.3 Cardiovascular Risk Factors in Individuals with Cerebral Palsy

Traditional cardiovascular disease risk factors, such as high blood pressure, high low-density lipoprotein cholesterol, diabetes, smoking and secondhand smoke exposure, obesity, unhealthy diet, and physical inactivity, were recognized from the Framingham Heart Study.[10] Several systemic and scoping reviews have been conducted on traditional cardiovascular risk factors and lipid metabolic markers in individuals with cerebral palsy.[11-13] These reviews showed that individuals with CP have a higher blood pressure and higher prevalence of hypertension compared to the general population,[13] reduced levels of physical activity throughout their lifespan,[11,12] and elevated levels of low-density lipoprotein cholesterol compared to reference values.[12]

In addition to traditional cardiovascular risk factors that have been studied for decades, non-traditional risk factors such as carotid artery intima-media thickness, arterial stiffness, endothelial dysfunction, and reduced artery diameter have emerged as important predictors of cardiovascular disease.[14-17] Especially in individuals with cerebral palsy, significant changes in non-traditional risk factors for cardiovascular disease were observed over 4 years, while no changes in traditional risk factors were observed.[30] Considering higher levels of low-density lipoprotein cholesterol observed in individuals with CP compared to reference values,[12] it is reasonable to emphasize the significance of closely monitoring non-traditional risk factors such as carotid intima thickness, artery stiffness, and endothelial function in this population. Another study showed that these non-traditional cardiovascular markers are evident in children and adolescents (10 - 18 yrs.) with cerebral palsy.[34] These findings indicate that non-cardiovascular risk factors tend to advance more rapidly and at an earlier age among individuals with cerebral palsy in comparison to the general population.

There were 9 studies in the area of arterial structures and function for individuals with CP. Six studies primarily focused on arterial structure and function in individuals with CP as cardiovascular risk factors. In summary, there were inconsistent findings regarding whether individuals with CP had distinct arterial structures and function and higher non-traditional cardiovascular risk factors compared to their healthy peers. In general, children with CP have a higher carotid intima-media thickness and lower flow-mediated dilation than healthy peers,[32,34] but there is lack of evidence to determine those trends in adolescents and adults with CP. These trends are likely related to the GMFCS level and age of individuals with CP.

In children with CP, two studies showed that children with CP have a higher average carotid intima-media thickness than controls,[32,34] while the other study observed no difference between them[33]. One study also demonstrated that children with CP have a lower absolute and relative flow-mediated dilation compared to controls,[34] while the other study did not find group differences in any flow-mediated dilation measurements between children with CP and controls.[33] These inconsistencies might come from the difference in the GMFCS level of the participants in these studies. Studies demonstrating differences in carotid intima-media thickness between children with cerebral palsy and controls included children with CP with GMFCS IV and V as participants.[32,34] One study in particular,[32] having 72% of the participants with cerebral palsy at GMFCS IV and V levels, showed a more pronounced difference than another study,[34] having only 31% of participants at those levels. The study[34] showing the difference in the brachial flow-mediated dilation, also included children with CP with GMFCS IV and V. The study[33] that did not observe the group difference in carotid intima-media thickness and the brachial flow-mediated dilation only had children with CP with GMFCS I and II as participants. These results suggest that the ambulatory status of participants might affect the difference in carotid intima-media thickness and brachial flow-mediated dilation in children with cerebral palsy. It is also plausible to infer that children with CP who are classified as GMFCS levels IV and V may exhibit increased cardiovascular risk factors in comparison to healthy peers. This result is consistent with the studies on general children, which showed that physical activity is related to arterial structure and function.[127,128] There is no study on carotid distensibility in children with CP.

Three studies compared the artery diameter of children with CP with healthy peers.[31,33,34] There is no group difference in carotid and brachial artery diameter in children with CP and controls.[33,34] It is important to note, however, that none of the studies reviewed provided information regarding the manual ability of the participants, which may affect the size of artery diameter. Therefore, it is important to be cautious when applying these findings to the larger CP population. In contrast, the diameter of the infrarenal abdominal aorta is smaller in children with CP than in controls.[31] While the diameter was not normalized with body surface area, it was noted that there was no discernible difference in body surface area between children with CP and controls. Consequently, it is suspected that the reduced exercise capacity resulting from activity limitations in children with CP may be responsible for the comparatively smaller infrarenal abdominal aorta diameter. Considering the fact that the infrarenal abdominal aorta is responsible for supplying blood to the lower body, these results suggest that the use of arteries related to the blood supply to the lower limb may be more appropriate for investigating the relationship between vascular structure, physical activity, and cardiovascular risks in children with cerebral palsy.

In contrast to the findings in children with CP, research on arterial structure and function in adolescents and adults with CP yielded conflicting results, albeit all studies on this topic we could find in this population were conducted by the same group of researchers.[30,73,129] Their study included adolescents and adults with CP but lacked a control group from the general population. Data were compared between different GMFCS level groups or sessions four years apart. A study conducted on adults with CP discovered no differences between the GMFCS I and II group and the GMFCS III-V group regarding carotid intima-media thickness, carotid distensibility, or absolute and relative flow-mediated dilation of the brachial artery.[129] However, another study found that adolescents and adults with CP classified as GMFCS I have a smaller carotid intima-media thickness than those classified as GMFCS II after adjusting for age,[73] although there were no noticeable differences in carotid distensibility, absolute flow-mediated dilation, or relative flow-mediated dilation. The inconsistent results observed in these studies could potentially be attributed to the age of the participants. Changes in arterial structure and function due to aging in individuals with CP tend to occur at a faster rate compared to the general population,[30] as mentioned later in this paper. It is crucial to take into account the age of the participants and make necessary adjustments. Neglecting this step will lead to inaccuracies and errors in the data analysis, ultimately undermining the validity and reliability of the study. They also found that moderate-to-vigorous physical activity is inversely related with carotid intima-media thickness in adolescents and adults with CP with GMFCS I and II.[73] This finding is consistent with the study of general population.[130]

In another study conducted by McPhee and team, carotid intima-media thickness, carotid distensibility, and brachial flow-mediated dilation were compared in adolescents and adults with CP over a period of 4 years.[30] The study revealed significant differences in carotid intima-media thickness, absolute flow-mediated dilation, and relative flow-mediated dilation between the baseline and follow-up assessments. No intersession difference in carotid distensibility was observed. While the impact of GMFCS

GMFCS level on adolescents and adults with CP did not seem to be significant, the findings from these studies highlight that non-traditional cardiovascular risks such as carotid intima-media thickness and brachial flow-mediated dilation are more sensitive indicators of significant changes in cardiovascular risks associated with aging in this population, compared to traditional cardiovascular risk factors. Additionally, McPhee and colleagues posited that over 4 years, carotid intima-media thickness and carotid distensibility in both adolescents and adults with CP exhibited higher rates of change compared to those in the general population. This statement seems reasonable, given that levels of physical activity have been shown to be inversely correlated with changes in carotid intima-media thickness over 8 years among the general population,[131] as well as the general activity limitations faced by individuals with CP. However, they only compared their data with the data published on the general population. Future research involving the control group is imperative to further advance our understanding of the vascular structure and function as cardiovascular risk factors in adolescents and adults with CP.

2.4 Physical Activity and Functional Tests of the Lower Limb in Children with Cerebral Palsy

2.4.1 Physical activity

The impact of physical activity on cardiovascular health and growth was demonstrated in several studies. Multiple studies suggest that regular physical activity is crucial for protecting children and teenagers against risk factors for cardiovascular disease. Engaging in higher levels of moderate-to-vigorous physical activity, which includes vigorous physical activity, has been linked to improved cardiorespiratory fitness[72,132] and a reduction in cardiometabolic risk factors.[65-67,72,133,134] Especially vigorous physical activity appears to be more important than moderate-to-vigorous physical activity.[68,135] Furthermore, studies showed that total activity count also has a similar or slightly more robust association with cardiometabolic disease risk factors compared to moderate-to-vigorous physical activity.[66,67] Conversely, sedentary physical activity is associated with an increased risk of chronic diseases[71,132,134] and impaired vascular function.[35] Additionally, maintaining higher levels of physical activity over the long term has been shown to result in improved physical fitness, reduced body fat, and better cardiovascular health in young individuals.[136] To promote optimal health, it is recommended that children and adolescents participate in approximately 60 to 90 minutes of moderate-to-vigorous physical activity each day,[65] including at least 20 minutes of vigorous physical activity.[137] Some studies also suggest moderate-to-vigorous physical activity is vital in promoting bone strength and overall bone health.[138-140] Gabel's study highlights the positive association between moderate-to-vigorous physical activity and bone strength, demonstrated by trabecular bone tissue volume and bone area.[139] Another study shows that even small amounts of daily physical activity, such as 5-20 minutes, can yield notable benefits for bone health.[140] Overall, these studies underscore the significance of physical activity in promoting and maintaining bone health across different age groups.

In studies involving children with disabilities, various methods were utilized to assess physical activity, including questionnaires, surveys, and interviews. However, an accelerometer physical activity monitor was the most frequently utilized method to objectively measure physical activity in ambulatory children.[141] Several studies have been conducted to measure physical activity in children with or without disabilities using various accelerometer monitors such as ActivPal,[142] Actiwatch,[143] RT3,[144] ActiGraph GT1M,[145] GT3X+,[2,146] and GT9X[147,148] monitors. Overall, these studies demonstrate the feasibility and reliability of using accelerometers to measure physical activity in children and adolescents with disabilities. However, it is important to be cautious when applying these monitors to specific populations, as using specific cut-points for the general population with different subpopulations, such as children with CP, may lead to misclassification of physical activity intensity.[144]

Several studies have shown that children with CP tend to have lower levels of physical activity compared to their typically developing peers.[72,149-152] The amount of time spent in moderate-to-vigorous physical activities was significantly lower in children with CP,[72,150,151] while sedentary behavior was higher.[72,150] Children with CP also accumulated less total activity and sustained bouts of moderate-to-vigorous activity.[72] Elevated blood pressure values were associated with reduced time in physical activity and increased time in sedentary behavior in children with CP, highlighting the importance of promoting physical activity to reduce cardiometabolic disease risk in this population.[153] Additionally, physical activity was related to the quality of life and happiness in individuals with CP.[154] The studies also revealed that physical activity levels decreased as functional abilities (i.e., Gross motor function level) declined in children with CP.[149,155,156] These findings underscore the need to promote and support physical activity in children with CP to improve their overall health and well-being.

2.4.2 Lateral Step-up Test

The lateral step-up test (LSUT) is a commonly used evaluation tool for assessing the functional muscle strength of the lower limbs. This test has been developed to evaluate the strength of the lower limb muscles, both concentric and eccentric, along with assessing the balance and proprioceptive sense of an individual.[157] It is particularly useful following a training protocol that emphasizes closed kinetic chain activities.[158,159] This test can provide valuable insights into the effectiveness of a training program designed to improve lower extremity strength and function.

The lateral step-up test is a highly reliable test that has been used and proven effective in a wide range of populations. These populations include healthy adults,[157,158] older adults who have experienced hip fractures,[160] patients who have undergone knee meniscectomy,[161] chronic stroke survivors,[162] and individuals with cerebral palsy.[163,164] Additionally, the validity of the LSUT in adolescents with CP has been confirmed by comparing it to other functional measurements such as the time up and go test, time up and down stairs, sit to stand, gross motor function measure (GMFM-88), and knee muscle strength.[163]

This test is performed in a closed kinetic chain, meaning the distal aspect of the extremity remains in contact with a stable object throughout the test. Clinical assessment of muscle strength is typically done manually or with a handheld dynamometer.[165] However, this method measures open kinetic chain muscle strength and does not adequately assess strength changes incurred via closed kinetic chain exercise.[158] Worrell[166] showed the inability of the isokinetic dynamometer to detect increases in quadriceps performance and recommended using closed kinetic chain testing to assess the function of the lower extremity. Closed kinetic chain assessments are often considered more functional, as they involve movements similar to those used in daily activities.[167] Also, lateral step-up creates more power and total work on the knee joint than forward step-up.[168] Rosenthal[159] showed that LSUT can be an alternative to isokinetic dynamometer testing, providing an objective clinical measure of work performance. The LSUT is an effective method for evaluating an individual's ability to perform daily activities that require strength, stability, and balance in the lower body.

The lateral step-up test is a commonly used assessment tool to measure the overall functional strength of lower limbs in children with cerebral palsy. The validity and reliability of the LSUT were verified in several studies. Chrysagis[163] demonstrated that the LSUT is a valid measure for lower muscle function, correlating with gross motor function measure (GMFM-88 (D & E)), time up and go test, time up and down stairs test, sit to stand test, and knee muscle strength within the appropriate range. Dallmeijer[169] found a moderate relationship between isometric strength and mobility capacity assessed by LSUT in children with CP, particularly hip abductor and knee flexor strength, but not in typically developing children. Kimoto[170] demonstrated that the LSUT score exhibits a positive correlation with gait speed and step length and a negative correlation with heart rate during walking. Verschuren[164] demonstrated excellent inter-tester reliability of the LSUT and poor-to-good reliability of hand-held dynamometry in children with CP. Several studies have been conducted to evaluate the efficacy of different training methods in improving muscle strength and gait function in children with cerebral palsy using the LSUT. These studies include strengthening exercises,[171] interactive home-based training,[172,173] group circuit exercise,[174] community-based exercise,[175] virtual and traditional golf training,[176] and investigation of prefrontal cortex hemodynamic activity during a test of lower extremity functional muscle strength.[177] They aimed to determine the feasibility and efficacy of these different forms of training to improve daily activities and overall functional lower limb strength in children with cerebral palsy.

The lateral step-up test is a widely used measure, but its procedure is not consistent. Verschuren was the first to verify the validity and reliability of the LSUT in children with CP. In his procedure, the test was performed with a 20 cm step, and the number of completed steps within 30 seconds was counted. Most studies have followed the procedure established by Verschuren.[169,173,176,178] However, some studies have used different step heights,[163,172,179] while others have used different test durations[174] or both different step heights and test durations.[175] Previous studies utilizing LSUT have been limited by using a single-step height, which fails to capture the variability and complexity of real-world settings. To address this issue, Licea and Modlesky proposed a progressive LSUT that incorporates multiple step heights.[177] By incorporating multiple steps in the test, it can better simulate the conditions where children with CP need to adapt to variable step heights. This incorporation will help the LSU test assess the lower limb function of children with CP more accurately, reflecting their activity capacity in a more realistic environment.

The lateral step-up test consisted of four 20-second trials with increased step height for each trial (0, 10, 15, and 20 cm). For the trials of 10, 15, and 20 cm, the foot of the more-affected leg of children with CP and the non-dominant leg of controls were placed on a step platform and considered the tested limb. The participants were told to lift one of their resting limbs, place it next to the tested limb, and then return it to its original position. They were then instructed to repeat this movement as many times as possible. Before each trial, the child was instructed to stand still for 20 seconds while focusing on an "X" sign placed 4.5 meters away at eye level. The lateral step-up test scores were calculated using a scoring system based on the height of the steps.

2.4.3 6 minute walk test (6MWT)

The 6MWT is a widely used submaximal exercise test in clinical settings, which measures the distance an individual can walk within a period of six minutes.[180] This test is commonly used to objectively assess functional exercise capacity in daily activities in patients with cardiopulmonary disease, neuromuscular disease, and other chronic diseases. In this simple test, which does not require any special or complicated equipment, the participant is asked to walk along a 30-meter corridor for six minutes, and the primary outcome measure is the distance covered during this time.

There were several suggestions of methods that can assess cardiorespiratory fitness without complex procedures and equipment. Balke[181] suggested a 15-minute best-effort run test as a simple field test for the assessment of the physical fitness of the aviator. Cooper[182] developed a 12-minute field performance test to evaluate the level of physical fitness of healthy individuals. Later, it advanced to a 12-minute walking test for assessing exercise tolerance in patients with chronic bronchitis.[183] However, walking for 12 minutes could be time-consuming for the investigator and exhausting for patients with respiratory disease. Thus, a 6-minute walk was suggested as an alternative method.[184] Guyatt[185] demonstrated that the 6MWT can be a measure of exercise capacity in patients with chronic heart failure also. Almost two decades after the adoption of the 6MWT, a review of functional walking tests concluded that the 6MWT is more reflective of activities of daily living than the other walk tests, such as 12-minute walk test, 2-minute walk test, self-paced walk test, and shuttle walk test.[186] The official guidelines for the 6MWT were established by the American Thoracic Society in 2002[180] and updated by the American Thoracic Society and European Respiratory Society in 2014.[187]

The six-minute walk test is widely used to measure the response to interventions and to assess the functional status of heart or lung disease patients. This test is beneficial for conditions such as lung transplantation, lung resection, lung volume reduction surgery, pulmonary rehabilitation, chronic obstructive pulmonary disease, pulmonary hypertension, heart failure, cystic fibrosis, peripheral vascular disease, fibromyalgia, and neuromuscular disease. Additionally, the six-minute walk test can predict morbidity and mortality for heart failure, chronic obstructive pulmonary disease, and primary pulmonary hypertension.[180,188]

The six-minute walk test has also demonstrated good validity and reliability in the pediatric population.[189-192]. It is a relevant assessment that mirrors daily physical activities[193] and the cardiorespiratory fitness of both healthy children[189] and those with various illnesses.[193,194] The six-minute walk test was used in children with severe cardiorespiratory disease,[195] cardiovascular disease, atherosclerosis, hypertension, obesity in youth,[196] cystic fibrosis, cerebral palsy, Duchene muscular dystrophy, obesity, end-stage renal disease, and pulmonary hypertension.[197]

The six-minute walk test is a submaximal exercise test because patients do not reach maximal exercise capacity, choose their own exercise intensity, and can rest during the test.[180] It measures the overall response of all the systems involved during exercise, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood, neuromuscular units, and muscle metabolism. It does not provide specific information on the function of individual organs and systems involved in exercise or the mechanism of exercise limitation. However, 6MWT distance showed good correlations with VO2max and VO2peak obtained in the laboratory in several studies.[192,194,198-200] These results showed that 6MWT can be an affordable and simple tool for assessing cardiorespiratory fitness. Moreover, this test is preferable for evaluating the functional exercise level for daily physical activities, as most everyday activities are performed at submaximal exertion levels. Compared to other walking tests, the 6MWD test is more representative of activities of daily living, making it a better choice for assessing one's functional capacity.[186]

The American Thoracic Society published guidelines for the 6MWT in 2002[180] and updated in 2014 with a subsequent joint with the European Respiratory Society.[187] The 6MWT should be performed on a flat, straight course with a hard surface of 30 m or more. Patients should be encouraged every 60 seconds using standardized phrases, while other words of encouragement and nonverbal prompts should not be used. The primary outcome to be reported is a 6-minute walk distance, calculated by recording the number of laps and any additional distance covered and rounding to the nearest meter or foot. The six-minute walk test is a reliable and safe test with very few complications. While there is no analysis of general patients, a large study of outpatients with chronic respiratory disease showed that the most common adverse event during the test was oxygen desaturation below 80%, occurring in only 5% of cases.[201] Additionally, adverse events occurred in only 6% of testing. Overall, the test is considered safe with minimal risks.

In patients with CP, the 6MWT indicates overall functional status rather than cardiorespiratory fitness. This is because the 6MWT performance requires the coordinated response of multiple body systems in this population, including the respiratory, cardiovascular, skeletal, nervous, and muscular systems.[180] It is uncertain whether the 6MWT can accurately measure cardiopulmonary fitness in individuals with CP[202] due to it being a submaximal exercise test, and several factors may affect the test results in people with CP. For instance, gait abnormalities caused by spasticity,[203] impaired balance,[204] and reduced muscle strength[205] may decrease walking speed during the 6MWT. Therefore, the 6MWT test results could be more influenced by potential limits to walking speed than by cardiorespiratory fitness. However, using 6MWT as a submaximal exercise test, which simulates daily life activities, is reasonable, as the Delphi study recommends.[206] In children with CP, it has been found that the 6MWT is a reliable tool for measuring walking capacity. The validity and reliability of this tool have been reported in children with CP,[194,207] and reference values have been established for both typically developing children[189,208] and children with CP.[209,210] Fitzgerald et al.[211] conducted a study in which they used 6MWT scores to measure walking abilities in children with CP across GMFCS levels I to III, as well as in typically developing children. The results showed significant differences in walking abilities between the groups. The six-minute walk test has been utilized as an adequate method to assess the effectiveness of various interventions aimed at improving the physical and functional abilities of children with CP. It was possible to identify progress in walking ability for individuals undergoing physiotherapy for 12-24 weeks using the 6MWT.[212] Additionally, the improvement after treadmill training for 4-8 weeks could be identified using the 6MWT.[213] One study was conducted using the 6MWT to observe the difference before and after the injection of botulin toxin. However, no changes were observed in the 6MWT results after five weeks from the injection.[214] Research have shown that robotic intervention has significantly improved walking capacity in children with CP, as detected by the 6MWT.[215,216] Studies have shown that interventions utilizing internet-based or intensive short-duration virtual reality techniques for 4 to 20 weeks can lead to significant improvements in the 6MWT distance, particularly when engaged in games involving gross motor activities.[217,218] The efficacy of walking abilities of WBV in children with CP can also be confirmed by improvements in 6MWT after at least four weeks of intervention.[219]

The six-minute walk test has significance in measuring the maximum distance that children can walk in 6 minutes. However, over the years, it has been discovered that it can also be a valuable tool in assessing changes in motor function and walking efficiency in children with cerebral palsy. This includes evaluating functional endurance, fatigue, posture, and stability after various interventions commonly used in this population of infants.

2.5 Body Surface Area and Allometric Scale

2.5.1 Body surface area (BSA)

The BSA is the measured or calculated surface area of a human body and is widely used to normalize a number of variables in pharmacology to assess the pharmacokinetics and in cardio physiology to assess the normal development of the cardiovascular system and physiological function. It is still controversial to use BSA as a normalization index,[220,221] especially in the overweight population[222] and children.[223] However, in pharmacology, the body surface area could be the best indicator of metabolic mass, because it combines gender-related scaling to liver size which plays a main role in pharmacologic metabolism.[224] Also, in cardio physiology, Sluysmans et al.[225] demonstrated that BSA could be a better indicator of the size of each of the cardiovascular structures than other anthropometric parameters such as age, height, or weight. Although Batterham et al.[226] suggested that fat-free mass is more appropriate to normalize the cardiac dimension data, reference standards for normality based on fat-free mass are lacking. Furthermore, several studies showed that BSA in children is significantly correlated with cardiovascular structural data such as diameters of the aorta,[227] coronary artery,[228] pulmonary artery,[229] carotid artery,[230] femoral artery,[231] popliteal artery,[115] and the size of heart.[232] Thus, it is reasonable to use BSA to normalize the artery diameter for assessing the rate of cardiovascular development in children with CP.

A number of studies used BSA for normalization. Naylor et al.[233] adjusted the diameter of brachial and femoral artery for BSA and found that this adjustment diminished differences in brachial artery diameters between athletes and controls, although adjusted femoral artery diameter was still significantly larger in athletes compared to controls. Lip et al.[234] found a group difference in the size of the coronary artery between Indo-Asians and Caucasians. However, the difference in size was not significant after correction for BSA. Roman[235] showed the size of the aortic root is influenced by gender. However, there are no differences in the size between genders when indexed for BSA. Not only the differences in the size of the artery but also the differences in cardiovascular responses to exercise, such as stroke volume and cardiac output between groups, disappeared after correction for BSA.[236] Also, BSA can be used for the prediction of artery diameters such as popliteal artery,[115] common femoral artery,[237] and coronary artery.[228] Sluysmans[225] described BSA as one of the fundamental driving factors in cardiovascular allometry, especially in children.

There are several formulas to calculate BSA. The oldest and most widely used formula for calculating BSA is the Du Bois formula.[238] However, the number of participants used to calculate the formula in their article is relatively small and their study included only one child, and also it could not reflect the changes in body composition due to the different diet and life patterns of these days. These limitations might be one of the causes of the previously mentioned controversy related to BSA. Thus, it could be inappropriate to use the Du Bois formula on children.[239] The formula developed by Haycock[239-241] could be a better option when studying the pediatric population. Because Haycock's formula is based on a considerably larger number of patients than Du Bois' formula and the patients are ranged from premature infants to adults.

Haycock formula: $\text{BSA} = 0.024265 \times Weight^{0.5378} \times Height^{0.3964}$

2.5.2 Allometric scale

Data from physiological or pharmacological testing are commonly confounded by anthropometrics and inappropriate to interpret when the data are not normalized. Allometric scaling removes the influence of anthropometrics such as weight, height, or BSA on physiological measurements, makes it possible to compare the data among children of different ages, genders, and sizes, and also facilitates to distinguish pathologic changes from normal physiologic growth.[242] Allometric scaling models use regression models to remove the potential confounders. There should be no significant correlation between the allometrically scaled dependent variables and the independent variable if the allometric scaling model is appropriate. These allometric models help to overcome the heteroscedasticity and skewness of variables and to reveal more unbiased relationships between variables. An allometric scale is generally used to assess pharmacokinetics assessment in pharmacology and to assess the normal development of the body and physiological function in physiology.

Nevill et al. suggested the power function ratio standard (Y/X^b) as a variable that normalized physiological measurements for individuals of various body sizes. It is derived from the allometric model ($\mathrm{Y} = \mathrm{a} \times X^b \times \epsilon$) that would remove the influence of the independent variable (X) on the dependent variable(Y). This allometric model can be analyzed using analysis of variance methods by taking logarithms of these variables. Also, as previously mentioned, the logarithmic transformation of variables could help to overcome the problems associated with linear regression, such as heteroscedasticity and non-normality. The log-log regression analysis provides the appropriate power for the normalized dependent variable independent from the independent variable.[243]

The allometric scale has been more widely used in physiological studies recently. Jaric et al. proposed allometric normalization as one of the standardized tests that normalize physical performance tests to body size.[244] This normalization for body size allows for comparison among individuals of different ages and genders and for diagnosis of pathological alteration. Several studies suggested scaling exponents for the normalization in various areas such as muscle strength,[245-247] peak jump force,[248] body circumferences,[249] body composition,[250] ambulatory behavior,[251] Maximal Oxygen Uptake,[252,253] and body mass.[254] Some studies also provide reference values independent from body size by using allometric scaling for diagnosis.[229,249,255,256]

An allometric scale can be applied to study based on children also. Wren showed that allometric scaling for children could provide more appropriate normalized variables than a simple normalization method that just divides by a variable itself.[257] Mahgerefteh et al.[258] also demonstrated that Allometric normalization based on BSA for cardiac structural measurement is feasible in children with various body sizes. Several studies previously mentioned based on children could get meaningful data by using allometric scaling.[251,253,255,256,259]

Physiological measurements require normalization for body size to appropriately compare the data between or within individuals and populations over time. The dilemma in power calculation for normalization is whether to perform the power calculation for the entire population to acquire general standards or for individuals to preserve the characteristics of each group. Heymsfield et al.[250] suggested both ways would be plausible, but he also pointed out that the power calculation for the entire group would introduce an independent variable bias in the independent variable normalized index of interest. Batterham and George[260] also showed that the allometric modeling based on entire groups could fail to provide the appropriate power for each group. Heymsfield et al.[254] made a separate regression model for each sex by race/ethnic group when they calculated the scaling exponent to demonstrate the difference between groups. Schuna et al.[261] also made separate regression models for each of the sex and race/ethnic groups to observe the differences between groups. Especially in children with CP, Wren and Engsberg[246] showed that allometric scaling from typically developing children failed to eliminate the influence of body mass in children with CP, but allometric scaling from children with CP could abolish the influence of body mass. Thus, if the purpose of the study focused on a demonstration of the difference between groups, or if the allometric scale model based on the entire group fails to fulfill the underlying model assumption, it is appropriate to make separate allometric scale models for each group.

CHAPTER 3

COMPROMISED SIZE AND ALTERED HEMODYNAMICS OF THE POPLITEAL ARTERY IN CHILDREN WITH CEREBRAL PALSY

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3.1 Abstract

Background: Children with cerebral palsy (CP) experience an earlier onset of cardiovascular disease and increased cardiovascular mortality. However, the state of the structure and hemodynamics of the arteries that supply blood to the lower limb of children with CP is unknown.

Objects: The aims of this study were to determine whether the size and hemodynamics of the popliteal artery are compromised in children with CP and to determine whether the expected effects persist after adjusting for fat-free soft tissue and lower limb muscle size.

Methods: Ambulatory children with spastic CP (5-11 y) and age-, sex-, and race-matched typically developing control children participated (n = 28/group). The popliteal artery diameter, blood flow velocity, mean shear rate, and oscillatory shear index, and gastrocnemius muscle volume were determined in both lower limbs using ultrasound. Muscle mass of leg was estimated using dual-energy X-ray absorptiometry. Resting systolic blood pressure, diastolic blood pressure, and heart rate were assessed using standard procedures.

Results: There were no group differences in physical characteristics. When controlling for BMI, diastolic blood pressure was higher in children with CP than controls ($p = 0.041$). Children in the bilateral CP subgroup had a higher systolic and diastolic blood pressure percentile than controls (both $p < 0.05$). Popliteal diameter of the more-affected lower limb in children with CP was smaller than controls. It was no longer different when statistically controlled for gastrocnemius muscle volume or leg muscle mass. When controlling for age, mean shear rate in the popliteal artery of the more-affected limb was higher in children with CP ($p = 0.44$), while there was no group difference in oscillatory shear index in either limb (both $p > 0.05$). The mean shear rate was higher in the popliteal artery of the more-affected limb in children with bilateral CP than children with unilateral CP ($p = 0.039$) and in the non-dominant limb in controls ($p = 0.004$). There were no group differences in blood velocity or blood flow in either limb in any subgroup (all $p > 0.05$).

Conclusion: Children with CP have a smaller popliteal artery with altered hemodynamics compared to typically developing children, which is linked to their small leg muscles. These observations provide insight into the non-traditional cardiovascular disease risk in children with CP. Further studies on the endothelial function and intima-media thickness of the popliteal artery in children with CP are warranted.

Keywords: Cerebral palsy, popliteal artery, arterial diameter, shear rate, cardiovascular disease risk factor

3.2 Introduction

Cerebral palsy (CP) is a non-progressive motor disorder.[3] Although the associated brain injury or malformation does not progress with time, degenerative changes appear much earlier in the lifespan than in the general population due to muscle weakness, impaired motor control, and activity limitations.[4] Concurrently, compared to neurotypical peers, an earlier onset of cardiovascular disease progression[5,6] and resultant increased cardiovascular disease-related mortality[7,8] is observed in those with CP. Cardiovascular disease is the third most common cause of death in individuals with CP,[9] and the mortality increases with the severity of their CP.[7] Therefore, assessing cardiovascular risk factors may provide insight into the pathophysiology of cardiovascular disease in individuals with CP. Many of the traditional cardiovascular disease risk factors that have been observed in the general population, such as high blood pressure, high low-density lipoprotein cholesterol, diabetes, smoking and secondhand smoke exposure, obesity, unhealthy diet, and physical inactivity,[10] are also present in individuals with CP;[11,12,262] however, they may be more profound and emerge earlier.[262-264] In addition to traditional cardiovascular risk factors, non-traditional risk factors are viewed as important predictors of cardiovascular disease in the general population.[14-17] Non-traditional risk factors, such as arterial structure and function, have the potential to improve the accuracy of cardiovascular disease prediction and the care for at-risk populations. Damage to the arteries underlies the pathophysiology of atherosclerosis, which is widely recognized as the primary cause of cardiovascular morbidity and mortality.[265] Atherosclerosis limits blood flow to tissue downstream by narrowing the lumen of arteries,[266] causes the formation of a thrombus,[267] provides a site for embolism,[268] or leads to the formation of an aneurysm.[269] Brachial[19] and carotid[17] artery diameter directly correlate with cardiovascular risk factors in adults, and greater carotid artery diameter is associated with a higher incidence of cardiovascular disease.[18] The increased artery diameter among individuals with cardiovascular risk factors may indicate impaired vasoregulation,[17,270] which is commonly associated with the progression of atherosclerosis.[271]

The observation that non-traditional risk factors display alterations, such as increased intima-media thickness and reduced flow-mediated dilation, sooner than traditional risk factors, suggests that non-traditional risk factors may offer earlier detection of cardiovascular disease in adults with CP.[30] Considering that elevated blood pressure has been reported in children with CP,[262] as well as a higher prevalence of pre-diabetes and dyslipidemia,[264] it is reasonable to explore the state of non-traditional risk factors in children with CP. However, studies examining the arterial structure and its hemodynamics in children with CP are lacking. A comprehensive literature search revealed only 5 studies on arterial structure and function in children with CP.[30-34] Among these studies, only 3 assessed arterial diameter,[31,33,34] and no study examined arterial hemodynamics. The studies that examined arterial diameter did not detect a group difference in carotid or the brachial artery diameter between children with CP and controls,[33,34] on the other hand, a small diameter of the infrarenal abdominal aorta has been reported in children with CP.[31] Consequently, it is suspected that the reduced exercise capacity resulting from activity limitations in children with CP[272] may contribute to the comparatively smaller infrarenal abdominal aorta diameter.[31] Considering that the infrarenal abdominal aorta supplies blood to the lower body and the deficits in fat-free soft tissue mass, a surrogate of muscle size, are greater in the lower limbs than the upper limbs of children with CP,[273] it is plausible that the vascular structure in the lower limbs is impaired and linked to the elevated cardiovascular risk in children with CP. This idea is supported by evidence that a reduction in physical activity primarily affects the arteries perfusing the limbs exposed to the greatest reduction in activity.[35] Children with CP, even those with a mild form, have low participation in physical activity.[274] Furthermore, the popliteal artery supplies the blood to the lower limb muscles including the hamstring, gastrocnemius, soleus, and plantar muscles, and represents the primary source of blood to the leg and foot.[36] Children with CP have substantially smaller muscles in the leg.[275,276] In addition, the deficits in arterial diameter in groups with extreme disuse, such as spinal cord injury,[277] are no longer present when the muscle size deficit is statistically controlled. Moreover, a direct relationship between the popliteal artery diameter and physical activity has been demonstrated in other populations.[38]

Several studies have reported that the conduit arteries of individuals with significant physical activity limitations, such as adults with spinal cord injury, have a high shear rate and a reduced diameter.[29,104,278,279] Shear stress is an important factor that determines the structure and function of arteries.[280] It is possible that the small arterial diameter is thought to offset the effects of reduced physical activity on artery function.[281] However, this was observed under a heated conditions. Similar observations are anticipated in children with CP. However, no studies have examined the structure or function of the popliteal artery in children, adolescents, or adults with CP. Therefore, assessing the popliteal artery diameter and its hemodynamics, which have been associated with the development of cardiovascular disease in children with CP, is warranted.

The aim of this study was to determine whether the size and hemodynamics of the popliteal artery are compromised in children with CP and whether the expected effects persist after adjusting for lower limb muscle size. We hypothesized that that popliteal artery would have a smaller diameter, a higher mean shear rate, and a lower oscillatory shear index compared to matched controls. We also hypothesized that the expected deficits in popliteal artery structure would be reduced or eliminated when statistically controlled for muscle size.

3.3 Methods

3.3.1 Participants

Children with spastic CP who were 5-11 years of age and ambulatory without an assistive device were recruited as part of a randomized controlled trial from Children's Healthcare of Atlanta, local schools, pediatric rehabilitation centers, and the Cerebral Palsy Foundation through the use of fliers and social media platforms. Typically developing children who were similar to children with CP in age (± 1.5y), sex, and race, between the 5th and 95th sex- and age-based percentiles for height and body mass, and not participating in high-level sports were recruited using the same methods and from the same areas as children with CP. Exclusion criteria for all participants included prior fracture in both femurs or tibias, currently taking bisphosphonates, orthopedic surgery within the last 6 months, a baclofen pump in the abdomen, and botulinum toxin treatment within the last year.

3.3.2 Anthropometrics

Height was measured to the nearest 0.1 cm using a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER) while participants were in an erect standing position. Body mass was measured to the nearest 0.1 kg using a digital scale (Detecto 6550, Cardinal Scale, Webb City, MO). Height and body mass were measured while children wore minimal clothing without shoes or braces.

3.3.3 Blood pressure and Heart rate

Resting systolic blood pressure, diastolic blood pressure, and heart rate were measured from the less-affected arm in children with CP and the dominant arm in controls with an oscillometric blood pressure device (Suntech 247; Suntech, Morrisville, NC). The appropriate size cuff was chosen based on the mid-arm circumference measured using a soft tape measure and placed over the brachial artery. Participants rested in a seated position with their backs supported and legs uncrossed for at least 5 minutes before two measurements were obtained at a 1-minute interval. Four measurements (twice in the morning and twice after 2 hours in the supine position) were obtained during a 2-day period. Mean arterial pressure was calculated as follows: Mean arterial pressure (mmHg) = Diastolic blood pressure (mmHg) + 1/3(Systolic blood pressure (mmHg) – Diastolic blood pressure (mmHg))

3.3.4 Artery structure and function, and muscle size

The diameter and blood flow velocity of the popliteal artery, and cross-sectional area and volume of the gastrocnemius were assessed in a prone position using ultrasound imaging (ACUSON S2000 Ultrasound System; Siemens Healthcare; Erlangen, GER) on the more- and less-affected limbs in children with CP and on the non-dominant and dominant limbs in controls after 30 minutes of bed rest. Video clips for estimating the diameter of the popliteal artery were recorded longitudinally for 18 seconds from the popliteal fossa using a 9L4 linear transducer in the cardiovascular mode. Video clips for assessing the blood flow velocity of the popliteal artery were recorded for 15 seconds at the same location using the same probe and mode with the pulse wave setting. All video clips were processed using FMD Studio within the Cardiovascular Suite software (Quipu srl; Pisa, Italy). FMD Studio has been validated, and its overall intra-day variability of artery diameter estimates is CV = 3 %.[282] All video clips from all the participants were collected and analyzed by one researcher. For analysis, the diameter of the popliteal artery was statistically controlled for gastrocnemius muscle volume or leg muscle mass[283] using allometric scaling.[242] To assess the hemodynamics of the popliteal artery, the mean shear rate, which reflects the shear stress to the vessel wall, was calculated using the following equation:

Mean shear rate (s^{-1}) = (4 × Blood flow velocity (mm/s)) / Artery diameter (mm)

The oscillatory shear index, indicating the degree of blood flow disruption, was calculated using the following equation:

Oscillatory shear index = |Retrograde shear rate (s^{-1}) | / (|Antegrade shear rate (s^{-1}) | + |Retrograde shear rate (s^{-1}) |)

Cross-sectional area of the gastrocnemius was determined using a 9L4 linear transducer in the musculoskeletal mode and the panoramic setting. Panoramic images of the gastrocnemius were collected at 25, 50, and 75 % of muscle belly length (2 sets of images per site), which was measured from the most proximal edge of the medial tibial condyle to the distal insertion of the medial gastrocnemius. The medial tibial condyle was located by palpating the knee, while the distal insertion of the medial gastrocnemius was located using ultrasound imaging. All images were processed using ImageJ (U. S. National Institutes of Health, Bethesda, Maryland, USA) by the same researcher. The cross-sectional area was determined by multiplying pixel size by the number of pixels within the outlined muscle region. The muscle volume of gastrocnemius was calculated by multiplying the average CSA of the three sites by the muscle belly length.

3.3.5 Leg muscle mass

Muscle mass of the leg was estimated using dual-energy X-ray absorptiometry (Horizon A, Whole Body Analysis; Hologic Inc., Bedford, MA). A whole-body scan was conducted using the standard procedure. When necessary, children were secured using the BodyFIX.[284] Fat-free soft tissue from the knee joint to ankle joint was determined from the whole-body scan. As previously described, leg muscle mass was estimated using fat-free soft tissue mass determined at the level of the middle-third of the tibia from the whole-body scan and a statistical model developed and cross-validated using children with CP (see below):[283]

Leg muscle mass (g) = 0.986 × Fat-free soft tissue mass (g) – 24.577

Because the model above is based on fat-free soft tissue mass from a different version of a dual-energy X-ray absorptiometry (Hologic Discovery W) than used in the present study, a calibration equation was developed using data from 14 individuals who were tested on each instrument within a 1-week period (Fat-free soft tissue mass Discovery W (g) = 1.063× Fat-free soft tissue mass Horizon A (g) - 0.486, $r^2 = 0.997$)

3.3.6 Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS 28. IBM, Armonk, NY, USA). Children with CP and controls were matched for age, sex, and race using the case-control matching option. Children with CP were divided into two groups according to their CP subtype for subgroup analysis (i.e., unilateral and bilateral CP). Variables were checked for normality by examining skewness, kurtosis, and by the Shapiro-Wilk test. Independent t-tests were performed to assess differences in height, body mass, and BMI compared to the 50th age- and sex-based percentiles. Analysis of covariance was used to determine group differences. Age was used as a covariate to statistically control for its effect on group differences. Given that BMI is a strong indicator of blood pressure[62] and BMI percentile is the standard measure for classifying overweight and obesity,[285] if there were group differences in BMI or BMI percentile, or if the BMI percentile varied from the 50th age- and sex-based percentiles, they were separately included as a covariate in an analysis of covariance model to ascertain any inter-group difference. Analysis of covariance and linear mixed effect models were used to determine group differences between the two CP subgroups and controls. Benjamin-Hochberg procedure was used for adjustment for multiple comparisons. The false discovery was set to 10%. The alpha level was set at 0.05.

3.4 Results

3.4.1 Physical characteristics

Twenty-eight children with CP and 28 age-, sex-, and race-matched controls participated in this study. Physical characteristics of the participants are reported in **Table 1**. There were no differences in age, height, height percentile, body mass, body mass percentile, BMI, or BMI percentile between children with CP and controls. Also, children with unilateral CP and children with bilateral CP were not different from controls in age, height, height percentile, body mass, body mass percentile, BMI, or BMI percentile. There was no difference in systolic and diastolic blood pressure, or mean arterial pressure between children with CP and controls (d = 0.26, 0.47, and 0.40, p = 0.344, 0.091, 0.147, respectively); however, when BMI percentile was statistically controlled, children with CP exhibited significantly higher diastolic blood pressure compared to controls (p = 0.041). Children with CP had a higher heart rate than controls (d = 0.60, p = 0.029).

In subgroup analysis, children with unilateral CP had a higher heart rate than controls (d = 0.71, p = 0.030), while children with bilateral CP had a higher systolic (d = 0.74, p = 0.014) and diastolic (d = 0.74, p = 0.039) blood pressure percentile than controls. Children with CP had lower gastrocnemius volume and leg muscle mass than controls in the more-affected leg (d = 0.93 and 0.83, p = 0.001 and 0.003, respectively) and lower gastrocnemius volume and leg muscle mass than controls in the less-affected leg (d = 0.62 and 0.50, p = 0.026 and 0.032, respectively). Compared to controls, children with unilateral CP had lower gastrocnemius volume and leg muscle mass in the more-affected leg (d = 0.85 and 0.68, p = 0.004 and 0.036, respectively). However, there was no significant difference in gastrocnemius volume or leg muscle mass (d = 0.35 and 0.23, p = 0.272 and 0.471, respectively) in the less-affected leg between children with unilateral CP and controls. Children with bilateral CP had lower gastrocnemius volume and leg muscle mass in both legs compared to controls (d range: 088 – 1.19, all $p < 0.05$).

Table 1. Physical characteristics, blood pressure, and muscle estimates of children with cerebral palsy (CP) and typically developing control children (Con)

	All CP (n = 28)	Unilateral CP (n=16)	Bilateral CP (n = 12)	Controls (n = 28)
Age (y)	8.8 ± 2.2	8.9 ± 2.1	8.6 ± 2.4	8.4 ± 2.2
Sex (boys/girls)	14/14	8/8	6/6	14/14
Race (White/Black/Asian)	24 / 3 / 1	14/2/0	10/1/1	24/3/1
GMFCS (I/II)	23/ 5	15 / 1	8 / 4	0 / 0
Height (m)	1.31 ± 0.14	1.33 ± 0.14	1.29 ± 0.15	1.32 ± 0.15
Height (%)	47 ± 33	54 ± 34	38 ± 33*	62 ± 26
Body mass (kg)	28.6 ± 9.1	28.6 ± 8.6	28.6 ± 10.0	29.5 ± 10.4
Body mass (%)	41 ± 30	41 ± 28	41 ± 35	54 ± 27
BMI (kg/m^2)	16.4 ± 2.7	16.0 ± 2.1	16. 9 ± 3.4	16.5 ± 2.5
BMI (%)	40 ± 30	36 ± 27	46 ± 33	50 ± 28
Systolic BP (mmHg)	106 ± 11	106 ± 12	107 ± 8	103 ± 8
Diastolic BP (mmHg)	65 ± 9	64 ± 8	66 ± 10	62 ± 6
MAP (mmHg)	79 ± 9	78 ± 9	80 ± 9	76 ± 6
Systolic BP (%)	73 ± 23	68 ± 28	81 ± 11*	68 ± 20
Diastolic BP (%)	68 ± 21	65 ± 22	72 ± 20*	59 ± 17
Heart rate (BPM)	89 ± 12*	89 ± 8*	90 ± 16	83 ± 9
Gastrocnemius volume (cm^3)				
More-affected/non-dominant	76.1 ± 30.3*	77.4 ± 29.9*	74.4 ± 32.1*	112.9 ± 48.4
Less-affected/dominant	88.1 ± 33.9*	98.2 ± 31.8	74.6 ± 33.0*	112.4 ± 45.0
Leg muscle mass (g)				
More-affected/non-dominant	236.8 ± 103.9*	228.3 ± 111.8*	189.3 ± 76.3.1*	295.2 ± 94.7
Less-affected/dominant	215.7 ± 101.9*	272.4 ± 109.6	198.8 ± 88.9*†	298.8 ± 98.7

Values are means ± SD, % reflects the percentile relative to age- and sex-based norms; GMFCS = gross motor function classification scale; BMI = body mass index; BP = blood pressure; MAP = mean arterial pressure; Gastrocnemius volume is estimated from ultrasound; Leg muscle mass is estimated from dual X-ray absorptiometry; *Different compared from controls, $p < 0.05$. †Different from children with unilateral CP, $p < 0.05$

3.4.2 Popliteal artery diameter

Popliteal arterial diameter of the more-affected leg in children with CP was smaller than that in controls ($d = 0.84$, $p = 0.001$; Figure 1). However, the difference was no longer statistically significant when normalized for gastrocnemius volume ($d = 0.44$, $p = 0.090$) or leg muscle mass ($d = 0.44$, $p = 0.081$). There was no difference in popliteal diameter of the less-affected leg in children with CP and the dominant leg of control children, regardless of normalization (all $p > 0.05$). Group comparisons of popliteal artery diameter are reported in Figure 1.

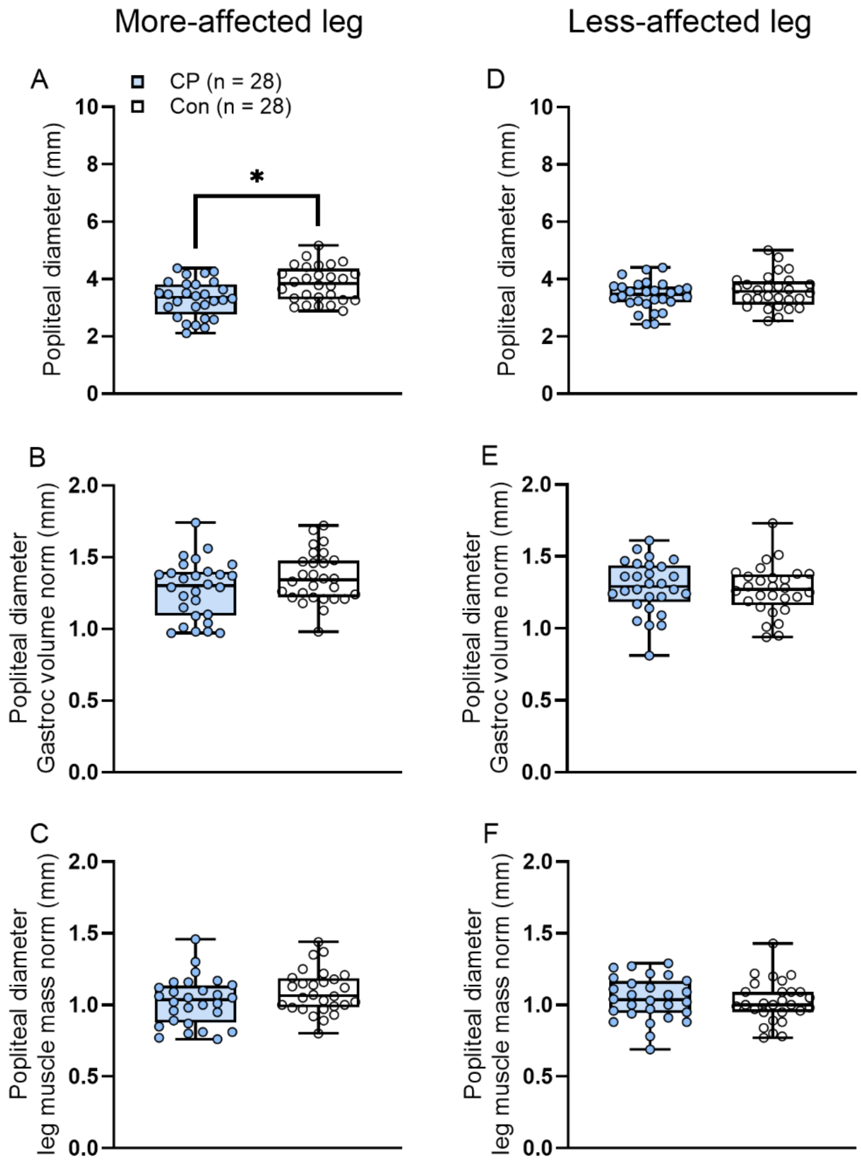

Fig. 1. Popliteal diameter in the more-affected leg of children with cerebral palsy (CP) compared to the non-dominant leg of typically developing controls (Con) and in the less-affected leg of children with CP compared to the dominant leg of Con. FFST norm = normalized with fat-free soft tissue; Gastroc volume norm = normalized for gastrocnemius muscle volume estimated from ultrasound; leg muscle mass = normalized for leg muscle mass estimated from dual-energy X-ray absorptiometry; *Group difference, $p < 0.05$.

Subgroup comparisons of popliteal artery diameter are reported in Figure 2. In the more-affected leg, popliteal artery diameter was lower in children with unilateral CP ($d = 0.66$, $p = 0.004$) and bilateral CP ($d = 1.10$, $p = 0.001$) than controls. No significant group difference was observed in the popliteal artery diameter when normalized for gastrocnemius muscle volume ($p = 0.152$) or leg muscle mass ($p = 0.178$).

In the less-affected leg, popliteal artery diameter was lower in children with bilateral CP than controls ($d = 0.68$, $p = 0.030$), but it was not different between children with unilateral CP and controls ($d = 0.06$, $p = 0.977$). The difference in children with bilateral CP was no longer statistically significant when normalized for gastrocnemius volume or leg muscle mass (all $p > 0.05$). There was no significant difference in popliteal diameter in either leg between children with unilateral CP or bilateral CP regardless of normalization (all $p > 0.05$).

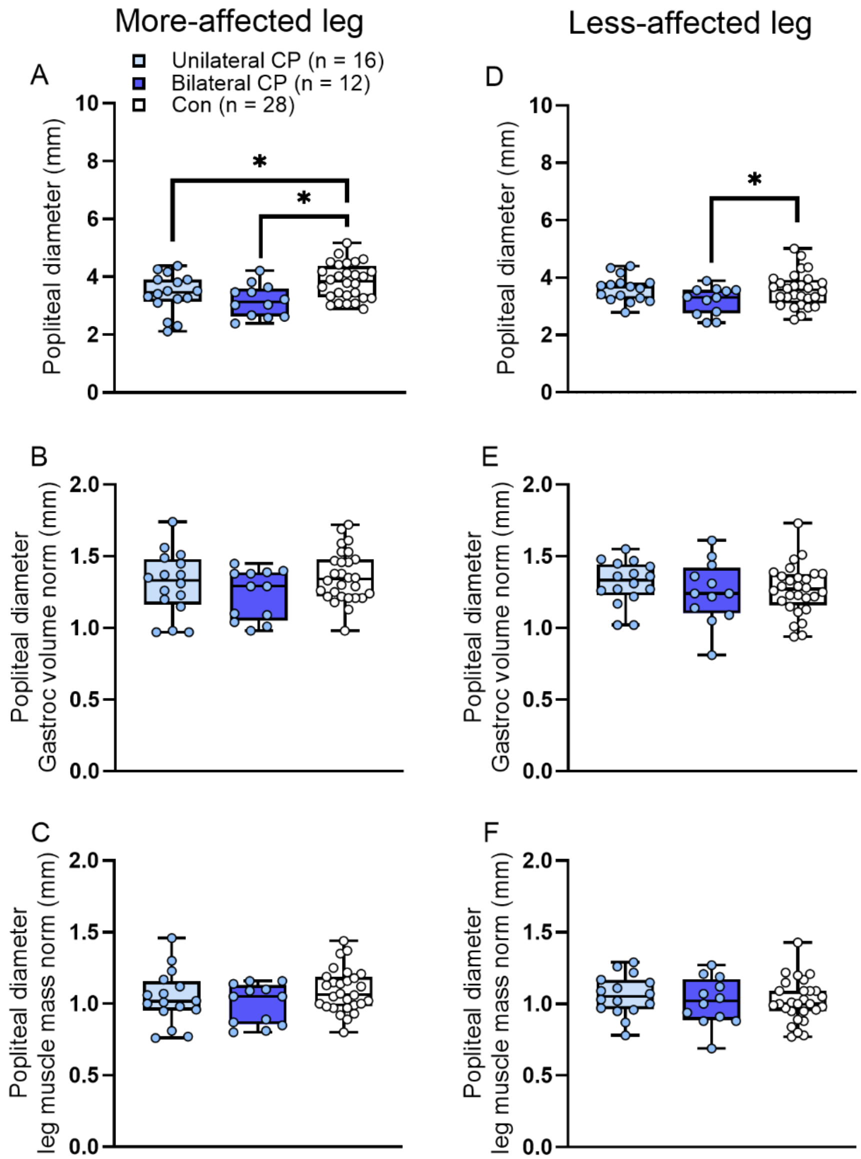

Fig. 2. Popliteal diameter in the more- and less-affected leg of children with unilateral and bilateral cerebral palsy (CP) compared to the non-dominant and dominant leg of typically developing controls (Con), respectively. Gastroc volume norm = normalized for gastrocnemius volume estimated from ultrasound; Leg muscle mass norm = normalized for leg muscle mass estimated from dual-energy X-ray absorptiometry; *Group difference, $p < 0.05$.

3.4.3 Blood velocity

Group comparisons of blood velocity in the popliteal artery are reported in Figure 3. There were no differences in positive, negative, or mean blood velocity between children with

CP and controls in either leg (*d* range = 0.08 – 0.47, *p* range = 0.09 – 0.76). Subgroup comparisons of blood velocity in the popliteal artery are reported in Figure 4. There were no differences in positive, negative, or mean blood velocity between children with unilateral CP, bilateral CP, and controls in either limb (*d* range = 0.02 – 0.73, *p* range = 0.17 – 0.34).

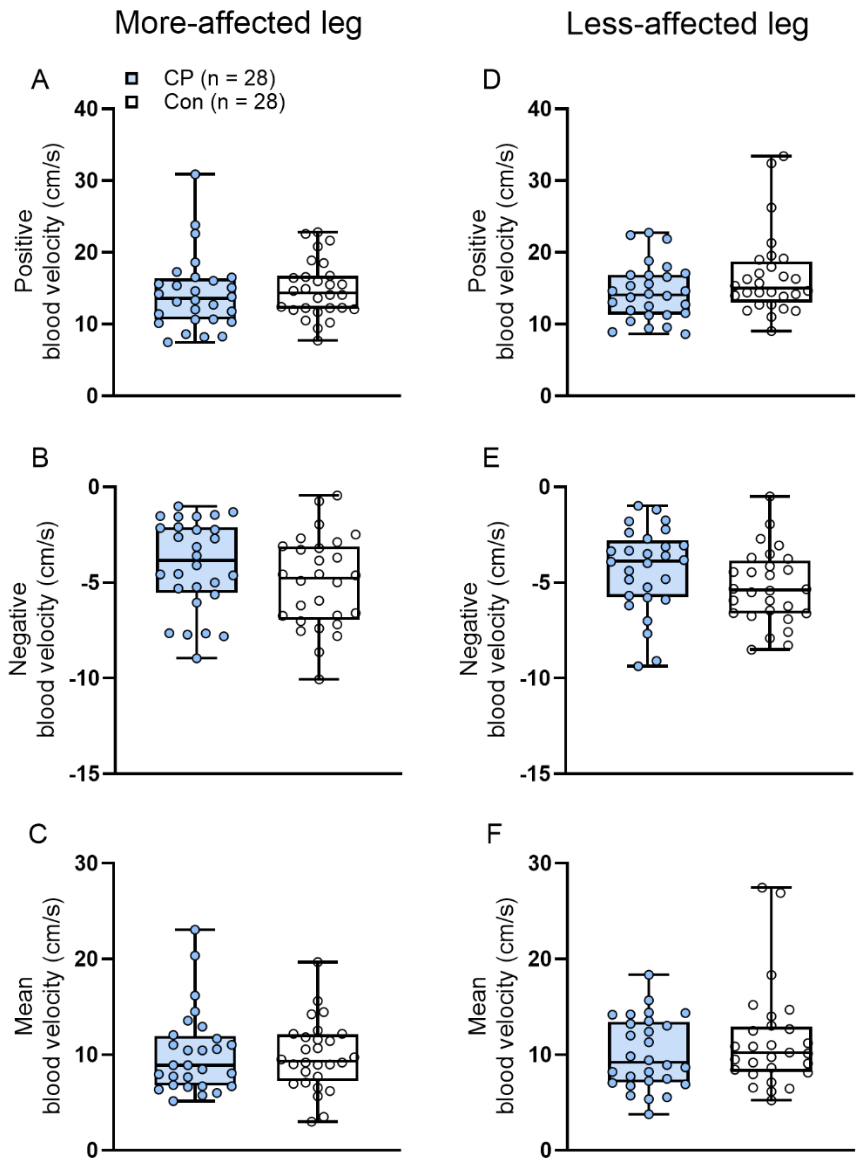

Fig. 3. Blood velocity in the more-affected leg of children with cerebral palsy (CP) compared to the non-dominant leg of typically developing controls (Con) and in the less-affected leg of children with CP compared to the dominant leg of Con.

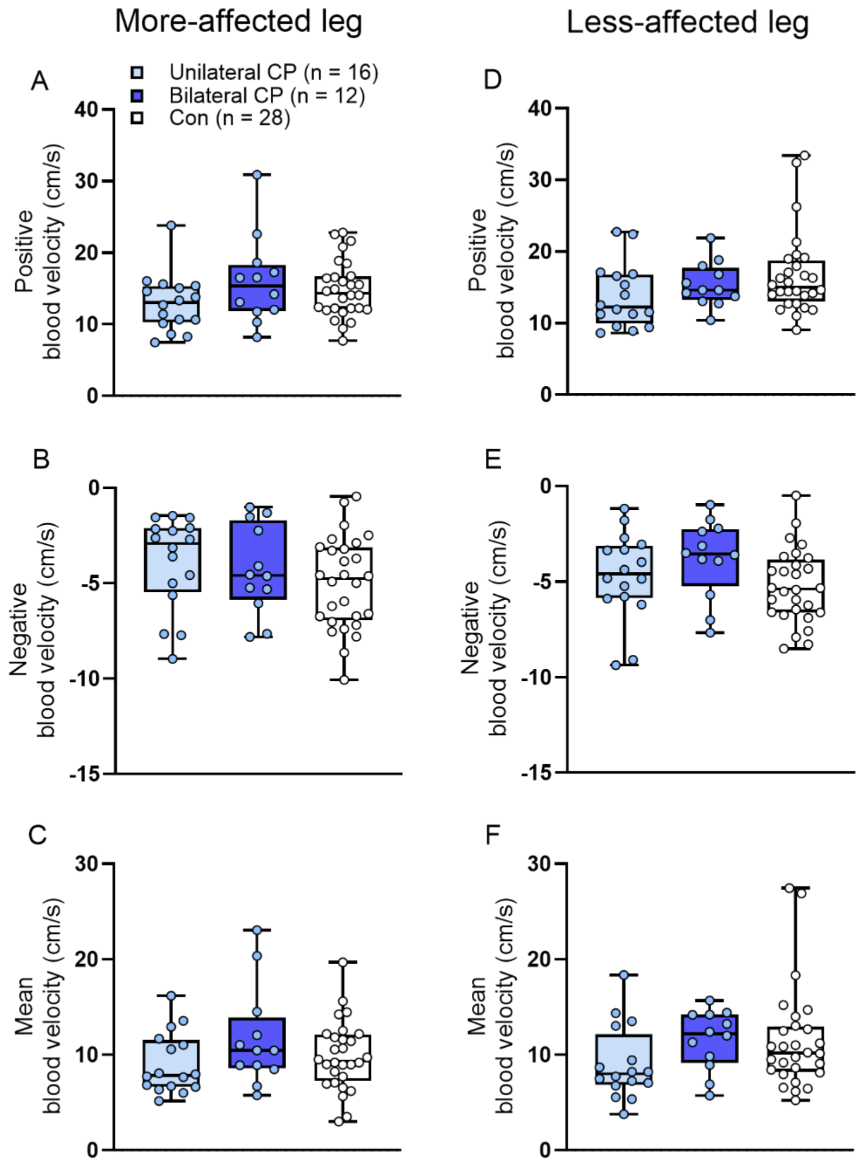

Fig. 4. Blood velocity in the more- and less-affected legs of children with unilateral and bilateral cerebral palsy (CP) compared to the non-dominant and dominant legs of typically developing control children (Con), respectively.

3.4.4 Mean shear rate & oscillatory shear index

Group comparisons of mean shear rate and oscillatory shear index in the popliteal artery are reported in Figure 5. Mean shear rate in the more-affected leg of children with CP was higher than that in the non-dominant leg of controls when age was statistically controlled (*d* = 0.46, *p* = 0.044), but there was no group difference in mean shear rate in the less-affected leg between children with CP and controls (*d* = 0.18, *p* = 0.604). There was no group difference in oscillatory shear index in the more-affected or the less-affected leg between children with CP and controls (*d* = 0.36 and 0.18, *p* = 0.189 and 0.506, respectively).

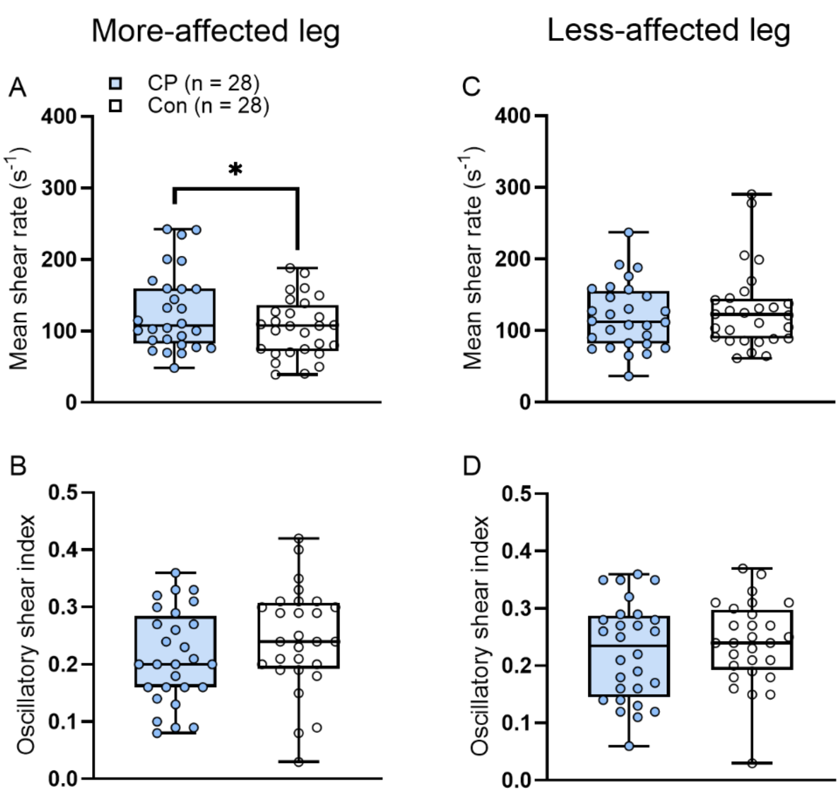

Fig. 5. Shear rate and oscillatory shear index in the more-affected leg of children with cerebral palsy (CP) and the non-dominant leg in typically developing control children (Con) and in the less-affected leg of children with CP compared to the dominant leg of Con; *Group difference, $p < 0.05$

Subgroup comparisons of mean shear rate and oscillatory shear index are reported in Figure 6. There was no significant difference in the mean shear rate of the popliteal artery in either leg between children with unilateral CP and controls ($d < 0.60$, $p > 0.05$). Mean shear rate of the popliteal artery in the more-affected leg in children with bilateral CP was higher than in children with unilateral CP ($d = 0.73$, $p = 0.039$) and in the non-dominant leg in controls ($d = 0.94$, $p = 0.004$). In the less-affected leg, children with bilateral CP had a higher mean shear rate than children with unilateral CP ($d = 1.11$, $p = 0.022$), but there was not a significant difference in mean shear rate between children with bilateral CP and controls ($d = 0.31$, $p = 0.294$). No significant group differences in oscillatory shear index were observed in any subgroup analysis (all $p > 0.05$).

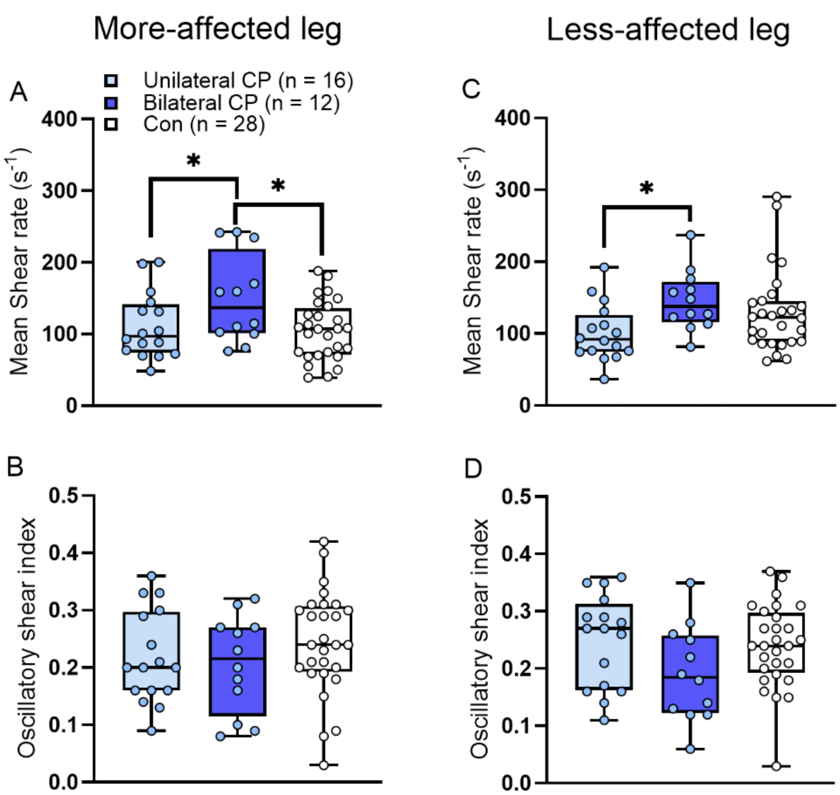

Fig. 6. Shear rate and oscillatory shear index in the more- and less-affected legs of children with unilateral and bilateral cerebral palsy (CP) compared to the non-dominant and dominant legs of typically developing controls (Con), respectively. *Group difference, $p < 0.05$

3.4.5 Blood flow

Group comparisons of blood flow are presented in Figure 7. Blood flow of the more-affected limb in children with CP was lower than that of the non-dominant limb in controls, but the difference was not statistically significant ($d = 0.47$, $p = 0.063$). There was also no group difference in blood flow in the less-affected leg of children with CP compared to the dominant leg in controls. There was no difference in blood flow in either limb in any subgroup analysis (all $p > 0.05$).

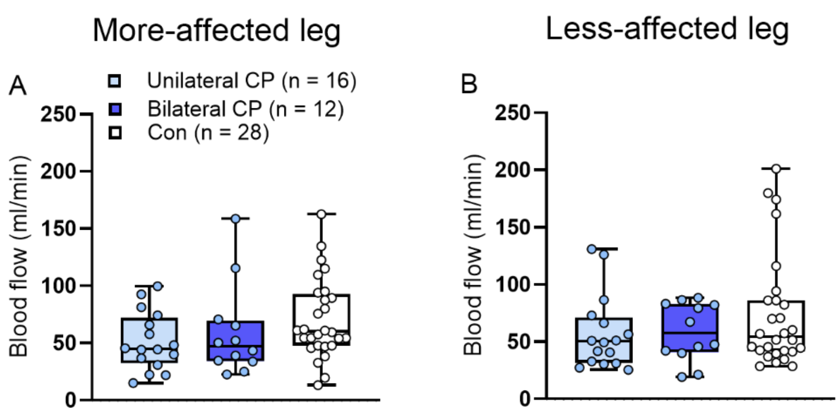

Fig. 7. Blood flow in the more- and less-affected legs of children with unilateral and bilateral cerebral palsy (CP) and the non-dominant and dominant limb of typically developing control children (Con), respectively.

3.5 Discussion

This is the first study to assess the size and hemodynamics of the popliteal artery in ambulatory children with CP. Consistent with our hypothesis, popliteal artery diameter was smaller in children with CP than in typically developing control children. The deficit was observed in the more-affected limb in children with unilateral CP, and in both limbs in children with bilateral CP. However, the size discrepancy was no longer present when it was statistically controlled for gastrocnemius volume or leg muscle mass suggesting that the deficit was primarily due to their smaller leg muscle size and reduced metabolic demands on the lower limbs. The other primary observation was that the mean shear rate was higher in the more-affected limb of children with CP. However, when subgroup analysis was performed, the higher shear rate was present in children with bilateral CP but not in children with unilateral CP. There is evidence that a higher shear rate induced through exercise or through other interventions can protect against atherosclerosis.[80,281] However, the relevance of a high shear rate during rest is unclear. The observations are important because cardiovascular disease is the third leading cause of death in individuals with CP[7] and understanding whether non-traditional markers contribute to the development of the disease is essential. Furthermore, it is important to understand whether physiological adaptations occur to offset disease progression.

Although the popliteal artery diameter was still smaller children with CP after statistically controlling for fat-free soft tissue, it was no longer present after controlling for gastrocnemius volume estimated using ultrasound or leg muscle mass estimated using dual-energy X-ray absorptiometry. This result is consistent with a previous report of a smaller femoral artery in individuals with spinal cord injury compared to controls without a spinal cord injury, which was no longer present after controlling the artery diameter for lower limb muscle volume.[277] Fat-free soft tissue estimates from dual X-ray absorptiometry have been suggested as a substitute for measuring the muscle mass of the appendicular in healthy adults[286] and children with CP.[287] However, the disappearance of the deficit upon adjusting the popliteal diameter for gastrocnemius muscle volume and leg muscle mass, but not fat-free soft tissue, raises uncertainties regarding the use of fat-free soft tissue as a substitute for assessing appendicular muscle mass in children with CP. It is important to consider that children with CP may have had additional contributing factors to their smaller popliteal diameter aside from their compromised muscle mass. Children with CP, especially bilateral CP, do not have the opportunity to grow in a typical manner during their development. Moreover, previous literature demonstrated that adolescents who experienced intrauterine growth restriction had a smaller popliteal artery diameter and abdominal aorta than typically developing adolescents, although they were healthy without any significant past medical history.[288] These observations could potentially extend to children with CP because 38 % of children with CP experienced intrauterine growth restriction.[289] However, it's important to interpret this information with caution, as the proportion of children with CP in the present study who experienced intrauterine growth restriction is unknown.

Children with bilateral CP had smaller popliteal arterial diameter when presented in absolute diameter or normalized to leg muscle size in the more- and less-affected legs than control children, while children with unilateral CP only had a smaller popliteal diameter in their more-affected leg. This result is expected because, in contrast to the more-affected leg, the less-affected leg in the children with unilateral CP is either unaffected or has limited affection and the gastrocnemius muscle volume of the less-affected leg in the children with unilateral CP did not differ from those of the dominant leg in controls. In children with bilateral CP, 33% (n = 4 of 12) were at GMFCS level 2, and the rest were at GMFCS level 1. On the other hand, in children with unilateral CP, only 6 % (n = 1 of 16) were at GMFCS level 2, and the rest were at GMFCS level 1. This unsurprisingly suggested that children with bilateral CP have more activity limitations compared to children with unilateral CP and controls which may have contributed to the small popliteal artery. These results suggest that the level of physical activity may have contributed to the small diameter of the popliteal artery. An inverse relationship between physical activity and artery diameter has been demonstrated in healthy populations[290] and individuals with spinal cord injury patients.[291] However, no such study has been done in children with CP. Further study on the relationship between physical activity and artery diameter in children with CP is warranted.

There were no significant differences in blood velocities between children with CP and controls. However, artery diameter was lower in children with CP. Because mean shear rate is established based on blood flow velocity and artery diameter, these results suggest that the elevated shear rate observed in children with CP was mainly due to the reduced arterial diameter rather than increased blood velocity. This observation contradicts the previous literature on adults with spinal cord injury, which reported that the increased mean shear rate in the popliteal artery was mainly due to higher mean blood velocity.[29] The difference between the observation in the previous literature and the present study may be attributed to the variations in the types of plegia among the participants. The previous literature specifically excluded individuals with hemiplegia but included those with paraplegia and tetraplegia. In the present study, half of children with CP had hemiplegia. Shear stress or shear rate plays a critical role in regulating the normal function of the vascular system and the pathophysiology that promotes atherogenesis[280]. High-shear stress induces atheroprotective endothelial gene expression, while low-shear stress stimulates the expression of an atherogenic phenotype.[87,281,292,293] Another study demonstrated that increased shear stress can prevent impairment in popliteal artery wall function caused by physical inactivity.[281] However, the shear rate in these studies was induced intentionally through exercise or interventions, whereas the shear rate in the present study reflects resting conditions. Therefore, caution should be given when interpreting the results of the present study. Our hypothesis was that children with CP would have a higher mean shear rate and lower oscillatory shear index compared to typically developing children due to their reduced artery diameter and activity limitation. Although there was significantly higher mean shear rate in the more-affected leg in children with CP than that in the non-dominant leg in controls, as expected, there was no significant difference in oscillatory shear index in either leg between children with CP or controls. This result is partially consistent with a previous study that reported reduced artery diameter and increased shear rate,[29,291] as well as a reduced oscillatory shear index[29,291] after inactivity due to spinal cord injury. Considering that all children with CP who participated in this study were ambulatory, it is possible that the smaller activity limitations of our participants were enough to mitigate a reduction in lower oscillatory shear index. However, whether a reduction develops as the children approach and enter adulthood requires further investigation.

Contrary to children with unilateral CP, children with bilateral CP exhibited a higher mean shear rate of the popliteal artery in the more-affected leg relative to controls. Children with bilateral CP also showed a higher mean shear rate of the popliteal artery in the less-affected leg than in children with unilateral CP. Given that children with bilateral CP have a greater activity limitation and smaller leg muscle mass than children with unilateral CP, these results suggest that a certain level of physical activity and restriction of artery diameter growth are required to demonstrate a higher shear rate. Further research is needed to understand the factors that contribute to the development of a higher resting shear rate in children with bilateral CP and its impact on the development of cardiovascular disease.

There are some limitations to consider in this study. First, the sample size was modest, although the effect size was large enough to detect a difference in artery diameter between children with CP and typically developing controls. Second, the limited sample size hindered comparisons based on GMFCS level, which is indicative of activity limitations. Only 5 out of 28 participants with CP were classified as GMFCS level 2. Third, although body surface area is a strong predictor of popliteal artery diameter,[115] according to previous literature, utilizing a formula based on body surface area may not be suitable for evaluating energy expenditure in children with CP.[294] Therefore, we used muscle size to normalize artery diameter, as it may provide a more effective means of evaluating the relevant physiological parameters. Lastly, we did not match the participants for height, which is positively associated with popliteal artery diameter.[115] Children with CP are generally shorter than typically developing children due to their hindered growth. Matching for height would have resulted in a comparison group that was different from the general population of children and made the difference in artery diameter even more distinct.

3.6 Conclusion

The results of the present study suggest that children with CP have a smaller popliteal artery diameter compared to typically developing controls. However, this difference was no longer present once adjustments were made to account for muscle size. This suggests that the reduced artery size is mainly due to their reduced leg muscles. Children with CP also showed a higher mean shear rate compared to controls. The relevance of these results is unclear, but it may reflect a disturbance in the hemodynamics of the popliteal artery.[73] in children with CP. Further studies on the endothelial function and intima-media thickness of the popliteal artery in children with CP are warranted.

CHAPTER 4

DIAMETER AND HEMYDYNAMICS OF THE POPLITEAL ARTERY AND THEIR RELATIONSHIP WITH PHYSICAL ACTIVITY AND FUNCTION IN CHILDREN WITH CEREBRAL PALSY

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4.1 Abstract

Background: The reason individuals with cerebral palsy are at increased risk of cardiovascular disease is still not apparent, although their limited mobility may play a role. The popliteal artery represents the primary source of blood to the lower limb. A relationship between the popliteal artery diameter, shear rate, and physical activity has been demonstrated in both those with limited mobility and the general population. However, no such study has been conducted in children, adolescents, or adults with CP.

Objective: The aim of this study was to determine the relationship between popliteal artery diameter and physical activity and function as well as between shear rate and physical activity and function in children with CP. The study hypothesized that physical activity and functional tests of the lower limb would be directly related to the popliteal artery diameter and negatively related to the shear rate in children with CP.

Methods: Children with spastic CP (n = 28) who were 5-11 years of age and age-, sex-, and race matched typically developing control children (n = 28) participated in the study. Physical activity was assessed using accelerometer-based monitors worn on the hip. Functional muscle strength was assessed using a progressive LSUT (LSUT).[177] 6-minute walk test (6MWT) was used to assess walking capacity. The diameter and blood flow velocity of the popliteal artery were estimated using ultrasound imaging. The shear rate was calculated.

Results: There were no group differences in physical characteristics. Children with CP had lower gastrocnemius volume than controls in the more- and less-affected legs ($p < 0.05$). Smaller popliteal artery diameter was observed in the more-affected leg of children with CP ($p < 0.05$). In bilateral CP, popliteal diameter was also smaller in the less-affected leg ($p < 0.05$).

Children with bilateral CP had a higher mean shear rate than those with unilateral CP or controls in the more-affected leg ($p < 0.05$). Children with CP had lower total physical activity counts, LSUT scores, and 6MWT distance than controls (all $p < 0.05$). Children with bilateral CP had significantly lower LSUT scores and 6MWT distance compared to children with unilateral CP and controls (all $p < 0.05$). Children with unilateral CP had lower LSUT scores than controls ($p < 0.05$) but did not differ significantly in other activity measures ($p > 0.05$). Popliteal artery diameter was positively related to 6MWT distance in children with CP ($p < 0.05$) but not in controls. Shear rate was inversely related to LSUT scores and 6MWT distance in children with CP (all $p < 0.05$), while no such relationships were observed in controls (all $p > 0.05$). In multivariate models, 6MWT was the most significant predictor of popliteal diameter in CP children, while LSUT score and 6MWT were predictors for shear rate (all $p < 0.05$).

Conclusion: Children with CP, especially bilateral CP, exhibit lower muscle volume, altered vascular measures, and lower physical activity compared to controls. Popliteal diameter and shear rate were related to lower limb function rather than physical activity in children with CP. Distance walked during the 6-minute walk test was a significant predictor of vascular measures in CP children. These results suggest that interventions to improve lower limb function, such as walking performance, may have beneficial effects on vascular health.

Keywords: Cerebral palsy, popliteal artery, shear rate, physical activity, 6-minute walk test, lateral step-up test

4.2 Introduction

Cerebral palsy (CP) is a neurological disorder arising from non-progressive brain insult or malformation during fetal development, birth, or early childhood.[3] Individuals with CP may experience degenerative changes much earlier in their lifespan than those in the general population due to various factors such as muscle weakness, impaired motor control, and activity limitations. Although the underlying brain injury or malformation does not progress over time, the effects of these factors can lead to the early onset of degenerative changes.[4] Furthermore, an earlier onset of cardiovascular disease progression[5,6] and resultant increased cardiovascular disease-related mortality[7,8] is observed in those with CP compared to healthy peers. Children with CP demonstrated higher visceral adiposity[264] and higher blood pressure.[262] Though their limited mobility may play a role,[11] the reason individuals with CP are at increased cardiovascular disease risk is still not apparent. For example, the relationship between physical activity and traditional markers of cardiometabolic disease, such as blood pressure, fasting blood glucose and low-density lipoprotein cholesterol, is inconsistent in children with CP, with some studies suggesting it weak or undetectable.[262,264]

Several studies have demonstrated the relationship between cardiovascular risk and physical activity in children with CP.[71,72] However, they only assessed the cardiovascular disease risk with traditional markers (i.e., blood pressure, body mass index, waist circumference) rather than the vascular structure and hemodynamics, although non-traditional risk factors such as carotid artery intima-media thickness and artery diameter reduction have emerged as important predictors of cardiovascular disease.[14-17] Some studies assessed the vascular structure and function in children with CP,[31,34,295] but they mostly focused on the brachial artery. The brachial artery would be sufficient to assess the general vascular function in children with CP. However, its location presents a drawback in evaluating the relationship with physical activity. This assumption is supported by the previous literature, which showed that a reduction in physical activity primarily affects the arteries perfusing the limbs exposed to the greatest reduction in activity.[35] Also, no group difference in carotid and brachial artery diameter between children with CP and controls was observed.[33,34] Another reported that children with CP have a smaller diameter of the abdominal aorta compared to healthy peers.[31] In this study, the researchers suspected the reduced exercise capacity resulting from activity limitations in children with CP may be responsible for the comparatively smaller abdominal aorta diameter. Due to the infrarenal abdominal aorta being responsible for supplying blood to the lower body, these results suggest that the use of arteries related to the blood supply to the lower limb may be more appropriate for investigating the relationship between vascular structure, physical activity, and cardiovascular risks in children with cerebral palsy. The popliteal artery supplies blood to the lower limb muscles and represents the primary source of blood to the leg and foot.[36] A relationship between the popliteal artery and physical activity has been demonstrated in both those with activity limitation[37] and the general population.[38] However, no such study has been conducted in children, adolescents, or adults with CP.

Shear stress plays a crucial role in maintaining vascular function, as consistent laminar shear stress protects against clotting,[20] preserves endothelial wall integrity,[21] and promotes the maintenance of vascular homeostasis.[22] Additionally, shear stress has the ability to regulate artery diameter by influencing the production of vasoactive mediators[23,24] and is a determinant of gene expression in endothelial cells.[25] In clinical settings, shear stress is typically measured by estimating whole blood viscosity and shear rate based on the blood flow velocity and internal diameter of the artery.[26] Engaging in physical activity can enhance vascular function and reduce the risk of cardiovascular diseases by inducing shear stress.[27] Despite increased cardiovascular risk,[28] shear rate is higher in those with spinal cord injury than in healthy adults,[29] which suggests that individuals with activity limitations may develop an atheroprotective hemodynamic environment to compensate for the adverse effect of inactivity on cardiovascular risk. However, no such study has been conducted in children, adolescents, or adults with CP.

Therefore, the aim of study was to determine if the artery diameter and hemodynamics of popliteal artery are related to physical activity and function.[33,34] The aim of this study was to examine the relationship between popliteal artery diameter and physical activity and function as well as between shear rate and physical activity and function in children with CP. The study hypothesized that physical activity and functional tests of the lower limb would be directly related to the popliteal artery diameter and negatively related to the shear rate in children with CP. Assessing the relationship between cardiovascular disease risk and physical activity in younger individuals with CP can offer valuable insights into the pathophysiology of cardiovascular disease in this particular demographic.

4.3 Methods

4.3.1 Participants

Children with spastic CP who were 5-11 years of age and ambulatory without an assistive device were recruited in a cross-sectional trial from Children's Healthcare of Atlanta, local schools, pediatric rehabilitation centers, and the Cerebral Palsy Foundation through the use of fliers and social media platforms. Typically developing children who were similar to children with CP in age (±1 yrs.), sex, and race, between the 5th and 95th sex- and age-based percentiles for height and body mass, and not participating in high-level sports were recruited using the same methods and from the same areas as children with CP. Exclusion criteria for all participants included prior fracture in both femurs or tibias, currently taking bisphosphonates, orthopedic surgery within the last 6 months, a baclofen pump in the abdomen, and botulinum toxin treatment within the last year.

4.3.2 Anthropometrics

Height was measured to the nearest 0.1 cm using a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER) while participants were in an erect standing position. Body mass was measured to the nearest 0.1 kg using a digital scale (Detecto 6550, Cardinal Scale, Webb City, MO). Height and body mass were measured while children wore minimal clothing without shoes or braces.

4.3.3 Ultrasound

The diameter and blood flow velocity of the popliteal artery were recorded in a prone position using ultrasound imaging (ACUSON S2000 Ultrasound System; Siemens Healthcare; Erlangen, GER) on both sides in children with CP and controls after 30 minutes of bed resting. The diameter of the popliteal artery was recorded for 18 seconds from the popliteal fossa longitudinally using a 9L4 linear transducer in the cardiovascular mode. The blood flow velocity of the popliteal artery was recorded for 15 seconds at the same location using the same probe in the cardiovascular mode with the pulse wave setting. All video clips were processed using FMD studio of the Cardiovascular suite (Quipu srl; Pisa, Italy). FMD Studio has been validated, and its overall intra-day variability for assessing artery diameter was CV = 3 %.[282] All video clips from all the participants were collected by one researcher. To assess the hemodynamics of the popliteal artery, the shear rate, reflecting the shear stress to the vessel wall, was calculated with the diameter and blood flow velocity. The Oscillatory Shear Index, indicating the degree of blood flow disruption, was also calculated with positive and negative shear rates.

4.3.5 Physical activity

Physical activity was assessed using the Actigraph GT9X activity monitor (Pensacola, FL). The monitor is equipped with a gyroscope, magnetometer, and triaxial accelerometer and measures acceleration between ± 8 g at a sampling rate of 30 to 100 Hz. The excellent validity of accelerometers to differentiate physical activity intensity in children with brain injury was demonstrated previously.[296] Participants wore the monitor on the hip of the more-affected side in children with CP and the non-dominant side in controls. Physical activity data was recorded for four days (i.e., three weekdays and one weekend day) while the participants wore the monitor continuously for 24 hours. Participants and their guardians were asked to remove the monitor only during bathing, showering, or swimming. These records were compared with the children's activity logs with assistance from their guardians, and the graphical output was generated using software provided by the manufacturer to verify the integrity of the physical activity data. If an issue was detected, the participant was asked to wear the monitor again. The total vector magnitude activity counts per day was reported as total physical activity. Physical activity intensity was determined for the hip physical activity data with Evenson's cut points[1,2] and used for analysis.

4.3.6 Lateral step-up test (LSUT)

Functional strength assessment of the more-affected leg (MAL) in children with CP and non-dominant leg in controls was done using a progressive LSUT.[177] The LSUT consisted of four 20-second trials with progressively increasing step heights (i.e., 0, 10, 15, and 20 cm). More than 30 seconds of rest was given between each trial. Participants were asked to stand with the less-affected leg (LAL) on the adjacent floor and the MAL on the step. They were instructed to keep their feet parallel and shoulder-width apart. They were also advised to maintain the position of the right foot in the same location on the steps for each level. Participants were instructed to move the LAL to the step and then bring the LAL back down to the floor by bending the MAL. One movement of the LAL from and back to the floor is considered one repetition of the test. Participants were asked to keep their hips, knees, and feet facing forward and avoid turning on the step during the test. Participants were then instructed to perform as many repetitions as they could at each level of the test without using any support. If support was required, any repetition that was not completed independently (e.g., assisted by an administrator's hand) was recorded but not included in their total step count. The test measured performance based on the total number of steps completed within a 20-second time limit. Before the test, the administrator demonstrated the lateral step-up technique visually and verbally and allowed practice attempts to ensure proper form.

4.3.7 6-minute walk test (6MWT)

Walking capacity was assessed using the 6-minute walk test (6MWT).[194,207] A loop of two 110-foot hallways and two 28-foot hallways was used as a track. Traffic cones were used to modify the turnaround points. All testing was conducted and assessed in the same looped corridor with a flat, obstacle-free surface. The children were instructed to walk as far as possible without running around a track for 6 minutes. Standard encouragements were provided every minute, such as "you are doing well, you have 5 minutes to go." and "Keep up the good work. You have 2 minutes left." The administrator followed and monitored the children during the assessment to ensure compliance. The distance covered during 6 minutes were recorded. Good validity and test-retest reliability of the 6MWT in individuals with CP has been demonstrated.[190,194,207]

4.3.8 Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS 28. IBM, Armonk, NY, USA). Children with CP and controls were matched for age, sex, and race using the case-control matching option. Children with CP were divided into two groups according to their CP type for subgroup analysis (i.e., unilateral and bilateral CP). Variables were checked for normality by examining skewness, kurtosis, and the Shapiro-Wilk test. Independent t-tests were used to determine group differences in normally distributed data. Mann-Whitney U tests were used to determine group differences in non-normally distributed data. Bivariate linear regression analysis was used to determine if the diameter and shear rate of the popliteal artery were related to physical activity and functional tests of the lower limb (LSUT and 6MWT). Multivariate linear regression was used to assess the relationships between popliteal diameter, hemodynamic responses to physical activity, LSUT scores, and 6MWT distance while accounting for age and gastrocnemius muscle volume. Interaction tests were conducted, and interaction terms were included in the models if they were statistically significant. The alpha level was set at 0.05.

4.4 Results

4.4.1 Physical characteristics

Twenty-eight children with CP and 28 age-, sex-, and race-matched controls participated in this study. Physical characteristics of the participants are reported in **Table 2**. There were no group differences in age, height, height percentile, body mass, body mass percentile, BMI, or BMI percentile between children with CP and controls or between children with unilateral CP and children with bilateral CP. Children with CP had lower gastrocnemius volume than controls in the more-affected- (d = 0.93, p = 0.001) and less-affected (d = 0.62, p = 0.026) leg. Compared to controls, children with unilateral CP had lower gastrocnemius volume in the more-affected leg (d = 0.85, p = 0.004) while there was no difference in the less-affected leg (d = 0.35, p = 0.272). Children with bilateral CP had lower gastrocnemius volume in the more-affected (d = 0.91, p = 0.006) and less-affected (d = 0.88, p = 0.013) leg compared to controls.

In the more-affected leg, popliteal arterial diameter was smaller in children with CP than in controls (d = 0.84, p = 0.001) and it was significantly smaller in children with unilateral CP (d = 0.66, p = 0.004) and bilateral CP (d = 1.10, p = 0.001) than controls. In the less-affected leg, popliteal artery diameter was smaller in children with bilateral CP than controls (d = 0.68, p = 0.030).

In the more-affected leg, children with bilateral CP had a higher mean shear rate compared to children with unilateral CP (d = 0.73, p = 0.039) and controls (d = 0.94, p = 0.004). In the less-affected leg, mean shear rate was higher in children with bilateral CP than those with unilateral CP (d = 1.11, p = 0.022), but not controls (d = 0.31, p = 0.294).

Table 2. Physical characteristics, muscle estimates, diameter, and shear rate of popliteal artery in children with cerebral palsy (CP) and typically developing control children (Con)

	All CP (n = 28)	Unilateral CP (n = 16)	Bilateral CP (n = 12)	Con (n = 28)
Age (y)	8.8 ± 2.2	8.9 ± 2.1	8.6 ± 2.4	8.4 ± 2.2
Sex (boys/girls)	14/14	8/8	6/6	14/14
Race (White/Black/Asian)	24 / 3 / 1	14/2/0	10/1/1	24/3/1
GMFCS (I/II)	23/ 5	15 / 1	8 / 4	0 / 0
Height (m)	1.31 ± 0.14	1.33 ± 0.14	1.29 ± 0.15	1.32 ± 0.15
Height (%)	47 ± 33	54 ± 34	38 ± 33*	62 ± 26
Body mass (kg)	28.6 ± 9.1	28.6 ± 8.6	28.6 ± 10.0	29.5 ± 10.4
Body mass (%)	41 ± 30	41 ± 28	41 ± 35	54 ± 27
BMI (kg/m^2)	16.4 ± 2.7	16.0 ± 2.1	16. 9 ± 3.4	16.5 ± 2.5
BMI (%)	40 ± 30	36 ± 27	46 ± 33	50 ± 28
Gastrocnemius volume (cm^3)				
more-affected leg / non-dominant	76.1 ± 30.3*	77.4 ± 29.9*	74.4 ± 32.1*	112.9 ± 48.4
less-affected leg / dominant	88.1 ± 33.9*	98.2 ± 31.8	74.6 ± 33.0*	112.4 ± 45.0
Popliteal diameter (mm)				
more-affected leg / non-dominant	3.31 ± 0.63*	3.41 ± 0.68*	3.18 ± 0.56*	3.84 ± 0.62
less-affected leg / dominant	3.43 ± 0.50	3.61 ± 0.45	3.20 ± 0.48	3.58 ± 0.59
Mean shear rate (s^{-1})				
more-affected leg / non-dominant	128 ± 56	111 ± 46	149 ± 62*†	105 ± 41
less-affected leg / dominant	120 ± 46	101 ± 40	146 ± 42†	129 ± 57

Values are means ± SD; % reflects the percentile relative to age- and sex-based norms; GMFCS = gross motor function classification scale; BMI = body mass index; Mean shear rate was measured from the popliteal artery; *Different compared with controls, p < 005, †Different compared with unilateral CP, p < 0.05.

4.4.2 Differences in physical activity and functional tests

Differences in physical activity and functional tests between children with CP and controls are shown in Figure 8 and between children with unilateral and bilateral CP and controls are shown in Figure 9. Compared to controls, children with CP had significantly lower total physical activity, light physical activity, LSUT score, and 6MWT distance (d = 0.54, 0.62, 1.42, and 0.66, respectively, all $p < 0.05$) and higher sedentary physical activity ($d = 0.65$, $p = 0.019$). They also showed a trend of lower moderate physical activity ($d = 0.47$, $p = 0.088$) and moderate-to-vigorous physical activity ($d = 0.43$, $p = 0.116$) than controls, but they were not statistically significant. Children with bilateral CP had significantly lower 6MWT distance than children with unilateral CP ($d = 1.20$, $p = 0.004$) and controls ($d = 1.18$, $p = 0.002$). They also had more sedentary activity, less light activity, and lower LSUT scores than controls (d = 0.78, 0.81, and 1.82, respectively, all $p < 0.04$). Children with unilateral CP had lower LSUT scores than controls ($d = 1.32$, $p = 0.001$), but there was no group difference in any other physical activity variables or 6MWT distance (all $p > 0.05$).

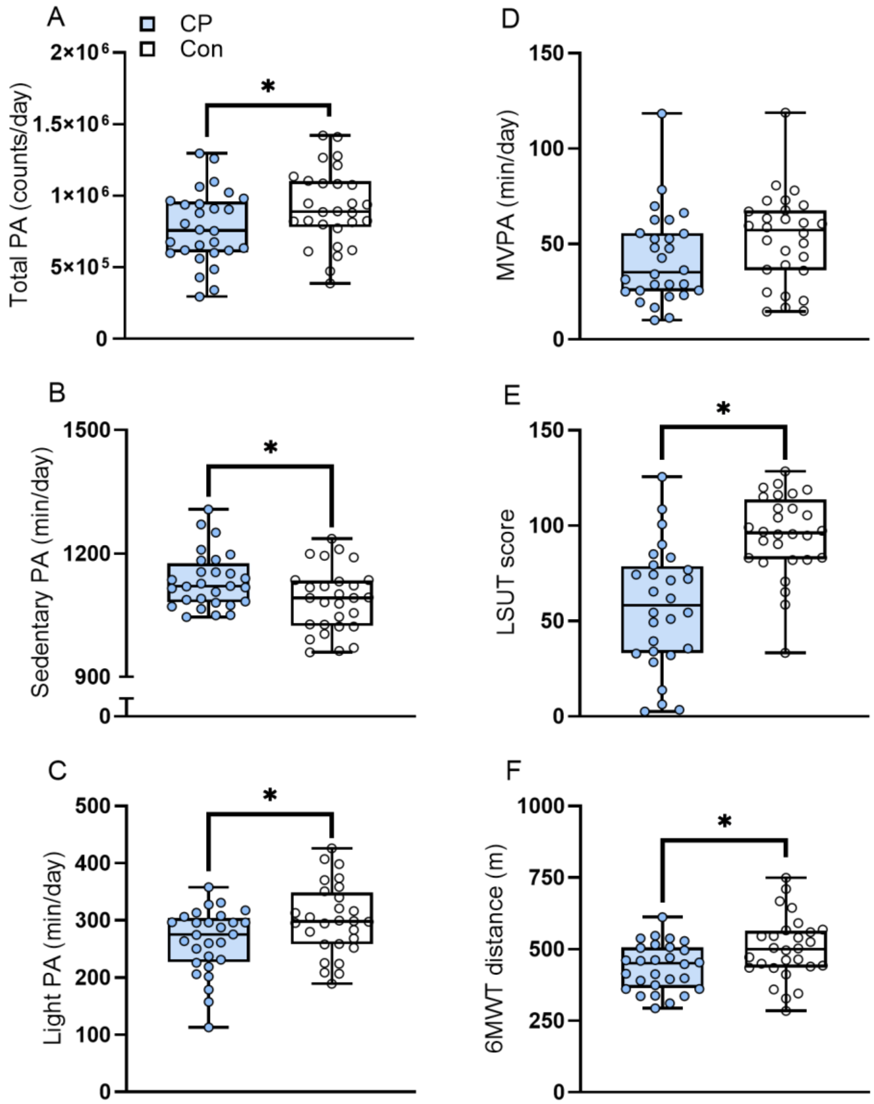

Fig. 8. Differences in physical activity and functional tests between children with cerebral palsy (CP) and typically developing controls (Con). Physical activity intensity was determined with Evenson's cut points,[1,2] PA: physical activity; MVPA: moderate-to-vigorous physical activity; LSUT = lateral step-up test; 6MWT = 6-minute walk test; *$p < 0.05$.

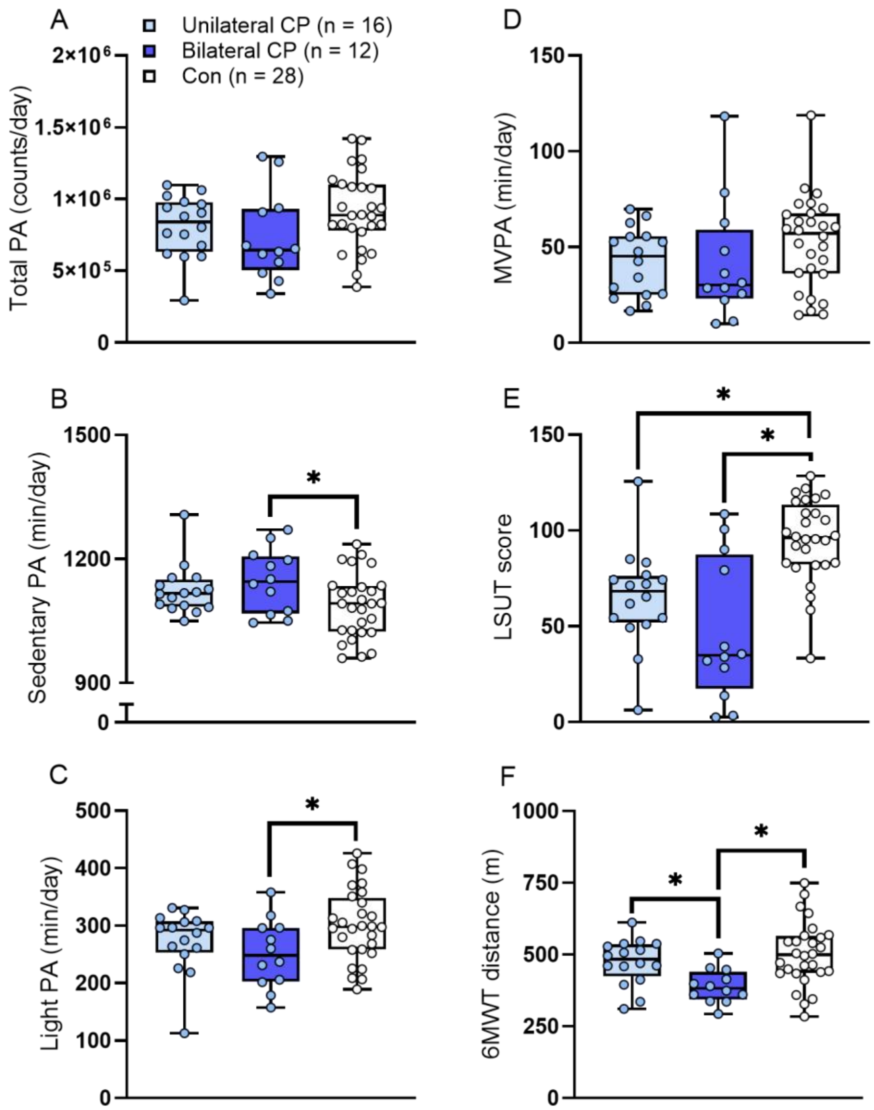

Fig. 9. Differences in physical activity and functional tests between children with unilateral and bilateral cerebral palsy (CP) and typically developing controls (Con). Physical activity intensity was determined with Evenson's cut points,[1,2] PA: physical activity; MVPA: moderate-to-vigorous physical activity; LSUT = lateral step-up test; 6MWT = 6-minute walk test; *$p < 0.05$.

4.4.3 Popliteal artery diameter and hemodynamics: relationships with physical activity, lateral step-up test, and 6-minute walk test

Scatter plots illustrating the bivariate relationships between artery diameter and shear rate with physical activity, LSUT, and 6MWT are shown in Figure 10. Linear regression analysis revealed no significant interactions between side and physical activity, side and LSUT score, or side and 6MWT for popliteal diameter (p = 0.969, 0.261, and 0.322, respectively) or shear rate (p = 0.895, 0.754, and 0.995, respectively). Thus, data from the more-affected and less-affected side were combined for analysis. In the combined sides, popliteal diameter was significantly and directly related to 6MWT in children with CP ($p < 0.001$), while it was significantly and directly related to LSUT scores in controls ($p < 0.01$). Mean shear rate showed a significant inverse relationship with both LSUT score and 6MWT in children with CP (p = 0.001 and 0.006, respectively), while no significant relationships were found in controls (all $p > 0.05$).

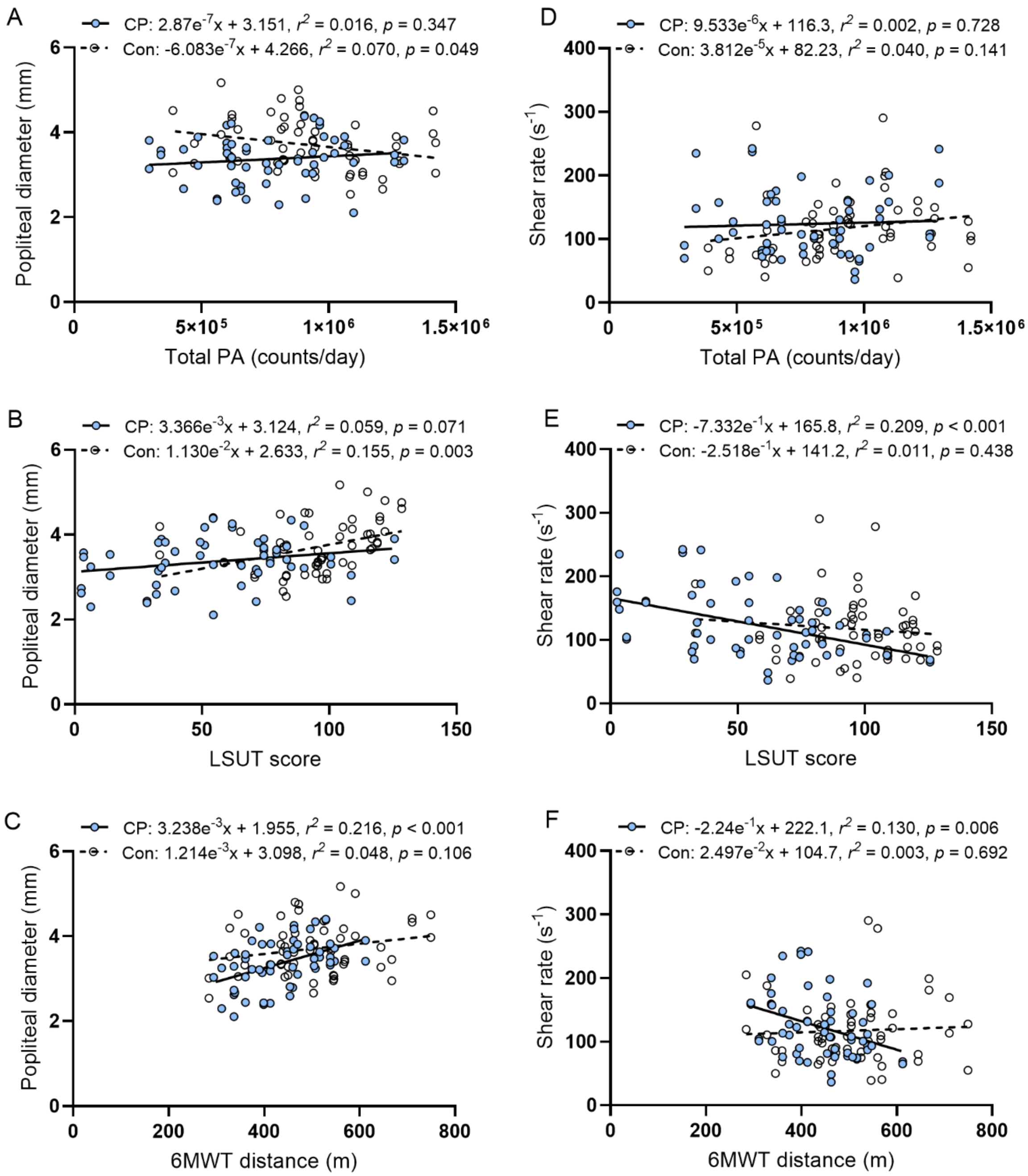

Fig. 10. Scatter plot demonstrating the relationship between diameter and shear rate of popliteal artery and physical activity and functional tests of lower limb in children with cerebral palsy (CP) and typically developing control children (Con). No significant side by physical activity, LSUT score, or 6MWT distance interaction were detected (all $p > 0.05$), therefore, the regression equation and associated regression line are reported for the combined sides (the more- and less-affected leg).

Multivariate linear regression models for the combined sides are presented in Table 3. When age and gastrocnemius muscle volume were included in the model with physical activity, LSUT score, or 6MWT, 6MWT was the only significant predictor of popliteal diameter in children with CP ($p = 0.005$). The LSUT score was a significant predictor of mean shear rate in children with CP when age and gastrocnemius volume were included in the model ($p = 0.032$). Age and 6MWT distance were also a significant predictor of shear rate ($p = 0.036$ and 0.035, respectively) in children with CP when they were included in a model with gastrocnemius volume. No significant predictors were detected for popliteal diameter or shear rate in controls (all $p > 0.05$). Gastrocnemius muscle volume was not a significant predictor in any models.

Table 3. Statistical models for predicting popliteal diameter and shear rate using age, gastrocnemius muscle volume, physical activity, and functional tests in children with cerebral palsy

Outcome measure	**Coefficients**	**β**	**t**	**SE**	***p***	**Std β**	**Model R^2, adj R^2**
Popliteal diameter							0.208, 0.163**
	Age (y)	0.060	1.231	0.049	0.224	0.227	
	Muscle volume (g)	0.004	1.355	0.003	0.181	0.248	
	Physical activity	$3.23e^{-7}$	1.098	<0.001	0.277	0.144	
							0.191, 0.144*
	Age (y)	0.044	0.921	0.047	0.361	0.165	
	Muscle volume (g)	0.006	1.704	0.003	0.094	0.318	
	LSUT score	$-4.39e^{-4}$	-0.158	0.003	0.875	-0.025	
							0.306, 0.266***
	Age (y)	0.050	1.171	0.043	0.247	0.191	
	Muscle volume (g)	0.003	0.869	0.003	0.389	0.149	
	6MWT (m)	0.001	2.950	<0.001	0.005	0.368	
Mean shear rate							0.189, 0.142*
	Age (y)	-8.025	-1.817	4.417	0.075	-0.340	
	Muscle volume (g)	-0.191	-0.659	0.290	0.513	-0.122	
	Physical activity	$1.72e^{-7}$	0.006	<0.001	0.995	0.001	
							0.258, 0.215**
	Age (y)	-6.248	-1.537	4.065	0.130	-0.264	
	Muscle volume (g)	0.017	0.061	0.280	0.951	0.011	
	LSUT score	-0.524	-2.200	0.238	0.032	-0.326	
							0.256, 0.213**
	Age (y)	-8.60	-2.153	3.996	0.036	-0.364	
	Muscle volume (g)	$-4.92e^{-4}$	-0.002	0.278	0.999	$-3.14e^{-4}$	
	6MWT (m)	-0.053	-2.168	0.025	0.035	-0.280	

Muscle volume = gastrocnemius muscle volume; Physical activity in counts/day; LSUT = lateral step-up test; 6MWT = 6-minute walk test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

4.5 Discussion

A novel observation in the present study, but consistent with our hypothesis, was that the small diameter of popliteal artery in children with CP was directly related to their functional capacity, as assessed by the 6MWT. These results indicate that children with CP who perform better on the 6MWT tend to have a larger popliteal artery diameter and suggest that the smaller diameter of the popliteal artery in children with CP might be due to their mobility limitation. Another novel observation, but consistent with our hypothesis, was that the high shear rate in the popliteal artery of children with CP was inversely related to their functional capacity, as assessed by the 6MWT, as well as the LSUT, which is more strongly related to functional muscle strength. This result suggests that the higher shear rate observed in children with CP may also be related to their mobility limitation, with a greater emphasis on more vigorous activities that require greater force generation. However, the relevance of the latter result is unclear. Interestingly, no relationships between the vascular measures and measures of functional capacity were observed in controls. Contrary to the study's hypotheses, neither the popliteal artery diameter nor the shear rate was associated with physical activity in children with CP. Controlling for age and gastrocnemius muscle volume, 6MWT emerged as the sole significant predictor of popliteal artery diameter in children with CP. This result suggests that children who exhibit the poorest lower limb function tend to have smallest popliteal arteries. Additionally, LSUT score and 6MWT distance were identified as significant predictors of mean shear rate of popliteal artery in children with CP, with age also playing a role in influencing shear rate. This observation underscores the importance of lower limb function in vascular function among children with CP. In contrast, no significant predictors for popliteal diameter or shear rate were found in controls, indicating that these relationships are particularly relevant to children with CP in the present study.

The 6MWT is an indicator of one's overall exercise capacity, encompassing the pulmonary, cardiovascular, and neuromuscular systems rather than focusing on a single limb in the general population.[68,132,152,180] The 6MWT is a widely used submaximal exercise test in clinical settings, which measures the distance an individual can walk within 6 minutes.[180] In children with CP, the 6MWT is a reliable tool for measuring walking capacity. The validity and reliability of this tool have been reported in children with CP,[194,207] and reference values have been established for both typically developing children[189,208] and children with CP.[209,210] Fitzgerald et al.[211] conducted a study in which they used 6MWT scores to measure walking abilities in children with CP across GMFCS levels I to III, as well as in typically developing children. The results demonstrated significant differences in walking abilities between the groups. In the present study, 6MWT distance was directly related to popliteal artery diameter and inversely related to the shear rate in children with CP. However, these relationships were not observed in controls. The present findings are partially inconsistent with prior literature, which demonstrated a weak correlation between 6MWT distance and the results of cardiopulmonary exercise testing in ambulatory individuals with CP.[202] The prior study concluded that the 6MWT is more appropriate for measuring walking capacity than cardiorespiratory fitness in persons with CP. Their conclusion appears plausible since the individuals with CP did not achieve VO2 peak during the 6MWT. However, the present study demonstrated that, in children with CP, 6MWT distance is related to artery diameter and the shear rate of the lower limb, which are directly related to cardiorespiratory adaptation. Thus, further studies are needed to determine the role of the 6MWT in the assessment of the cardiorespiratory system in children with CP.

Unexpectedly, physical activity, which was represented by total physical activity counts, did not relate to the diameter and shear rate of popliteal artery in the present study. This is inconsistent with previous studies which showed that physical activity can have a positive impact on cardiovascular health by affecting the artery diameter in general populations[136] and patients with spinal cord injuries.[297] Considering that one study was conducted in a healthy population without mobility limitations,[136] and the other involved an intervention of cycling training in patients with spinal cord injury,[297] it is possible that habitual physical activity alone may not be sufficient to induce changes in the diameter and shear rate of the popliteal artery in children with CP. Unlike the controlled exercise interventions or the active, mobility-unrestricted individuals in these previous studies, children with CP have mobility limitations that may limit the vascular adaptations typically associated with physical activity.

The lateral step-up test is a widely utilized assessment tool for measuring the functional muscle strength of the lower extremities.[157] The LSUT score showed a direct relationship with popliteal artery diameter in the control group, while no such relationship was observed in children with CP. It is possible that the relationship between the LSUT score and the popliteal artery diameter in controls is due to muscle size, as muscle strength is related to the LSUT score,[162] and muscle size is related to artery diameter.[298] The absence of a relationship between the popliteal diameter and the LSUT score in children with CP implies that other variables might affect the diameter of the popliteal artery in children with CP apart from muscle size. Limited mobility or growth disturbance before birth could be the factors for the smaller artery observed in this study. The results of the present study highlight the need for further exploration of such factors to better understand the underlying pathophysiology of CP. In children with CP, there was an inverse relationship between the LSUT score and shear rate. However, this relationship was not present in the control group. This result is consistent with a previous study in individuals with spinal cord injury, which reported that an increase in shear rate was accompanied by a decrease in muscle volume.[277,299,300]

There are some limitations that should be mentioned. First, our sample size was relatively small, although it is large enough to detect the relationships between artery diameter, shear rate, physical activity, and functional tests. Second, the physical activity data from the monitor worn on the ankle may be more appropriate for analyzing the relationship between physical activity and variables from the popliteal artery due to their proximity. However, there are no established cut points for activity intensity for monitors worn on the ankle.

4.6 Conclusion

The observation that popliteal diameter and shear rate were related to lower limb function but not physical activity in children with CP suggests that lower limb function is a better indicator of cardiovascular risk in children with CP than physical activity. The results also suggest that the 6MWT distance is a significant predictor of vascular measures in CP children. Lastly the results suggest that the limited functional capacity of children with CP may suppress popliteal artery growth.

CHAPTER 5

INTER-ARM DIFFERENCE IN BLOOD PRESSURE MEASUREMENT IN CHILDREN WITH CEREBRAL PALSY

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5.1 Abstract

Background: Children with CP are at higher risk for elevated blood pressure and hypertension, which is linked to their higher levels of adiposity and is consistent with their limited exercise capacity. Children with CP often have one side of the body that is more affected. This impact may affect blood pressure assessments, typically taken from one side of the body. However, the appropriate arm to measure blood pressure in children with CP remains uncertain.

Objective: The aim of the study was to determine whether blood pressure measurements are consistent across arms in ambulatory children with CP. We hypothesized that blood pressure in the more-affected arm would be higher than in the less-affected arm and would result in a greater number of children classified as having elevated blood pressure or hypertension.

Methods: Ambulatory children with spastic CP (5 - 11 years) and age-, sex-, and race-matched typically developing children were included in the study (n = 33/group). The gross motor function classification level, the Modified Ashworth Scale, and the type of CP were assessed. Resting systolic and diastolic blood pressure were measured in both arms.

Results: There was no significant inter-arm difference in blood pressure in children with CP or controls (all $p > 0.05$). Among 33 children in each group, 4 children with CP (all with bilateral CP) demonstrated a pathologically significant difference in inter-arm blood pressure (>10 mmHg) and none in the controls. There was no significant inter-arm difference in blood pressure in children with unilateral CP (n = 19) and bilateral CP (n = 14; all $p > 0.05$). In children with CP, 21 % were classified as having elevated blood pressure and 12 % as having hypertension when using the blood pressure percentile from the more-affected arm. When using the less-affected arm, a similar proportion were classified as having elevated blood pressure (18 %) and hypertension (15 %). In the control group, 9% were classified as having elevated blood pressure and 6% as having hypertension when using the non-dominant arm. When using the dominant arm, a similar proportion was classified as having elevated blood pressure (9 %) and hypertension (3 %). Children with CP had higher diastolic blood pressure, mean arterial pressure, and diastolic blood pressure percentile on the more-affected arm and higher diastolic blood pressure on the less-affected arm (all $p < 0.05$) compared to controls.

Conclusion: In most instances, blood pressure measurements taken from the more- and less-affected arms yield similar results in children with CP. However, almost 30 % of children with bilateral CP exhibited a pathologically significant inter-arm difference (>10 mmHg). Thus, assessing blood pressure in both arms may be necessary in children with bilateral CP.

Keywords: Cerebral palsy, blood pressure, inter-arm difference

5.2 Introduction

Children with CP are at a higher risk for elevated blood pressure, which is associated with their excess adiposity, especially in the viscera,[262] and is consistent with their limited exercise capacity.[301] Whether it is associated with low participation in physical activity is unclear.[71] Researchers demonstrated that blood pressure in ambulatory persons with CP depended on GMFCS level.[73] However, it is likely linked to mobility limitation, as there is evidence that those who walk without limitations have lower blood pressure than those with limitations.[71,73,153] Furthermore, the higher prevalence of hypertension in children with CP compared to the general population continues to adulthood,[13] which is also reported in the generation population.[59-61] Blood pressure is a traditional measure demonstrating cardiovascular disease risk. Early identification and intervention of high blood pressure in children may reduce cardiovascular disease risk in the future.

One concern about blood pressure measurement in children with CP is the arm used to represent blood pressure status best. In the general population, for consistency and comparison with standard tables and for the possibility of coarctation of the aorta, the right arm is preferred for repeated measurements of blood pressure.[39,40] On the other hand, in patients with hemiparesis, the nonparetic arm is preferred because the muscle tone in the paretic arm could influence blood pressure.[41] Additionally, a rating greater than 2 on Modified Ashworth Scale is associated with significantly higher blood pressure in patients with hemiplegia.[42] In children with CP, there is no widely accepted rule yet. In a review about blood pressure in adults with CP,[13] only half of the included studies measured blood pressure in the least affected or unaffected arm. Others used the left or right arm regardless of the affected side. Although it is generally recommended to measure blood pressure in the right arm for consistency with standard reference tables,[40] considering that spasticity could affect blood pressure measurements in children with CP, it may be more practical to measure blood pressure in their less-affected arm. In a recently published study,[262] elevated blood pressure was detected in children with CP when it was assessed on the less-affected arm. The aim of the present study was to determine whether blood pressure measurement is consistent across arms in ambulatory children with CP and to assess its impact on the diagnosis of elevated blood pressure or hypertension. We hypothesized that the blood pressure in the more-affected arm would be higher than in the less-affected arm leading to a greater number of participants diagnosed with elevated blood pressure or hypertension.

5.3 Methods

5.3.1 Participants

Ambulatory children with spastic CP (5 - 11 y) were recruited as part of a randomized controlled trial from the Children's Healthcare of Atlanta, local schools, pediatric rehabilitation centers, and the Cerebral Palsy Foundation using fliers and social media platforms. Typically developing children who were matched with children with CP in terms of age (± 1.5y), sex, and race, falling within the 5th to 95th percentiles for height and body mass based on sex and age, and not engaged in high-level sports, were recruited as controls during the same period, using the same methods and from the same locations as children with CP. All participants were required to meet certain exclusion criteria, including no current use of medicine that could affect blood pressure, no orthopedic surgery or injury within the last 6 months, no presence of a baclofen pump in the abdomen, and no botulinum toxin treatment within the last year. Parents or guardians provided written informed consent, and children provided oral assent before testing. The study was approved by the institutional review board at the University of Georgia.

5.3.2 Anthropometrics

Height was assessed in an erect standing position with a stadiometer (Seca 217; Seca GmbH & Co. KG., Hamburg, GER) to the nearest 0.1 cm. Body mass was assessed with a digital weight scale (Detecto 6550, Cardinal Scale, Webb City, MO) to the nearest 0.1 kg. Height and body mass were measured while children wore minimal clothing without shoes or braces. Body mass index (BMI) was calculated based on height and body mass.

5.3.3 Gross Motor Function Classification System (GMFCS)

Gross motor function was assessed by a health professional using the GMFCS.[54] Ratings of I and II on the GMFCS indicate gross motor independence, such as independent ambulation, with limited ability of speed, balance, and coordination. GMFCS III demonstrates the use of assistive devices, and GMFCS IV and V reveal wheelchair-empowered mobility. In this study, only those children who were GMFCS levels I and II participated, suggesting all of the participants could ambulate independently.

5.3.4 Modified Ashworth Scale

The Modified Ashworth Scale was performed on children with CP to assess the spasticity of their lower limbs. This five-point ordinal scale (0: normal muscle tone, 1: Slight increase in tone, 2: More marked increase in tone, but limb easily flexed, 3: Considerable increase in tone, 4: limb rigid in flexion) measures resistance encountered during manual passive muscle stretching.[302] The same rater performed all assessments on ankle plantar flexor muscles bilaterally using standardized procedures.

5.3.5 Type of cerebral palsy

The type of CP (i.e., unilateral and bilateral) was assessed by a health professional. Children with unilateral CP were affected on one side of the body, and children with bilateral CP were affected on both sides of the body.

5.3.6. Blood pressure measurements

Resting systolic and diastolic blood pressure were measured from both arms in children with CP and control with an oscillometric blood pressure device (Suntech 247; Suntech, Morrisville, NC). The appropriate size cuff was chosen based on the mid-arm circumference and placed over the brachial artery. Participants rested in a seated position with their back supported and legs uncrossed for at least 5 minutes before two measurements were obtained at a 1-minute interval. Four measurements (twice in the morning and twice after 2 hours of bed rest) were obtained during a 2-day period. Evidence shows that the first blood pressure measurement may be higher than subsequent measurements.[40] However, in our preliminary analysis, there were no significant differences between blood pressure measurements in this study. Therefore, an average of 4 measurements was used in the data analysis. Mean arterial pressure was calculated as follows: Mean arterial pressure = diastolic blood pressure + 1/3(systolic blood pressure – diastolic blood pressure)

The occurrence of hypertension and elevated blood pressure was identified according to the American Academy of Pediatrics' 2017 clinical practice guideline.[40] Age- and sex-based pediatric blood pressure reference charts were used to calculate the blood pressure percentile. Systolic or diastolic blood pressure more than or equal to the 90th percentile but less than the 95th percentile was classified as elevated blood pressure and systolic or diastolic blood pressure more than or equal to the 95th percentile was classified as hypertension.

5.3.7 Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS 28. IBM, Armonk, NY, USA). Variables were checked for normality by examining skewness, kurtosis, and the Shapiro-Wilk test. Independent t-tests were used to determine group and subgroup differences in normally distributed data. Mann- Whitney U tests were used for non-normally distributed data and Tanner stage. One-sample t-tests were conducted to determine if height, body mass, and BMI were different from the 50th age- and sex-based percentiles. A group-by-arm two-way analysis of variance with repeated measures on arm was used to determine if there were inter-arm differences in BP and if the differences were consistent across groups. A CP subtype-by-arm two-way analysis of variance with repeated measures on arm was used to determine if the suspected inter-arm differences in BP in children with CP were consistent across children in the unilateral and bilateral CP subtypes. Fisher's exact tests were used to determine if the prevalence of elevated blood pressure or hypertension was different between groups. Intraclass correlation coefficient test was conducted to verify the consistency of measurements from the more-affected and less-affected arm. Values are presented as mean ± SD unless stated otherwise. Alpha level was set at 0.05. Cohen's *d* of 0.2, 0.5, and 0.8 were used to represent small, medium, and large effect sizes, respectively.[303]

5.4 Results

Thirty-three children with CP and 33 age-, sex-, and race-matched controls participated in this study. Of the 33 children with CP, 19 were classified as unilateral CP, and 14 were classified as bilateral CP. The physical characteristics of the groups are reported in Table 4. There were no group differences in age, sex, race, height, body mass, or BMI (all $p > 0.05$). However, there was a trend for a lower BMI percentile in children with CP ($p = 0.100$).

Table 4. Physical characteristics of children with cerebral palsy (CP) and typically developing children (Con).

Variable	CP (n = 33)	Unilateral CP (n = 19)	Bilateral CP (n = 14)	Con (n = 33)
Age (y)	8.7 ± 2.2	8.9 ± 2.2	8.4 ± 2.2	8.5 ± 2.2
Sex (M/F)	18/15	10/9	8/6	18/15
Race (White/Black/Asian)	29/3/1	18/1/0	11/2/1	29/3/1
Height (m)	1.30 ± 0.14	1.32 ± 0.13	1.28 ± 0.14	1.32 ± 0.15
Height (%)	48 ± 34	53 ± 33	41 ± 36	59 ± 28
Body mass (kg)	27.8 ± 7.1	27.8 ± 6.9	27.8 ± 7.8	29.1 ± 10.3
Body mass (%)	43 ± 29	39 ± 24	48 ± 35	54 ± 29
BMI (kg/m^2)	16.1 ± 2.3	15.7 ± 1.9	16.8 ± 2.8	16.6 ± 2.6
BMI (%)	39 ± 31	32 ± 26	49 ± 34	52 ± 30
GMFCS (I/II)	28/5	18/1	10/4	-

Values are mean ± SD. % reflects the percentile relative to sex- and age-based norms; BMI: body mass index; Gross Motor Function Classification System.

Inter-arm comparisons of blood pressure measurements in children with CP and controls are reported in Figure 11. There was no significant inter-arm difference in blood pressure in children with CP (all $p > 0.05$) or controls (all $p > 0.05$). Out of the 33 children in each group, 4 children with CP (12%) exhibited an inter-arm blood pressure difference (>10 mmHg), all of which had bilateral CP. None of the children in the control group showed such a difference.

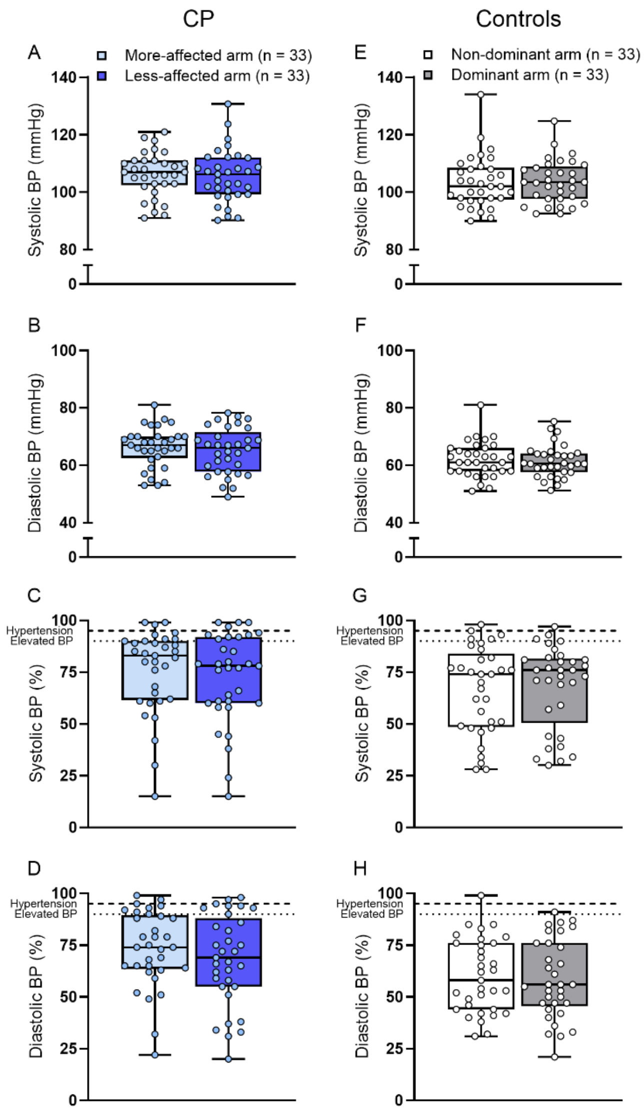

Fig. 11. Comparison of inter-arm blood pressure in children with cerebral palsy (CP) and typically developing control children (controls). BP: blood pressure; Systolic or diastolic blood pressure more than or equal to the 90th percentile but less than the 95th percentile was classified as elevated blood pressure, and systolic or diastolic blood pressure more than or equal to the 95th percentile was classified as hypertension

Inter-arm comparisons of blood pressure measurements in children with unilateral CP and bilateral CP are reported in Figure 12. No significant inter-arm difference in blood pressure was observed in either CP subtype (all $p > 0.05$).

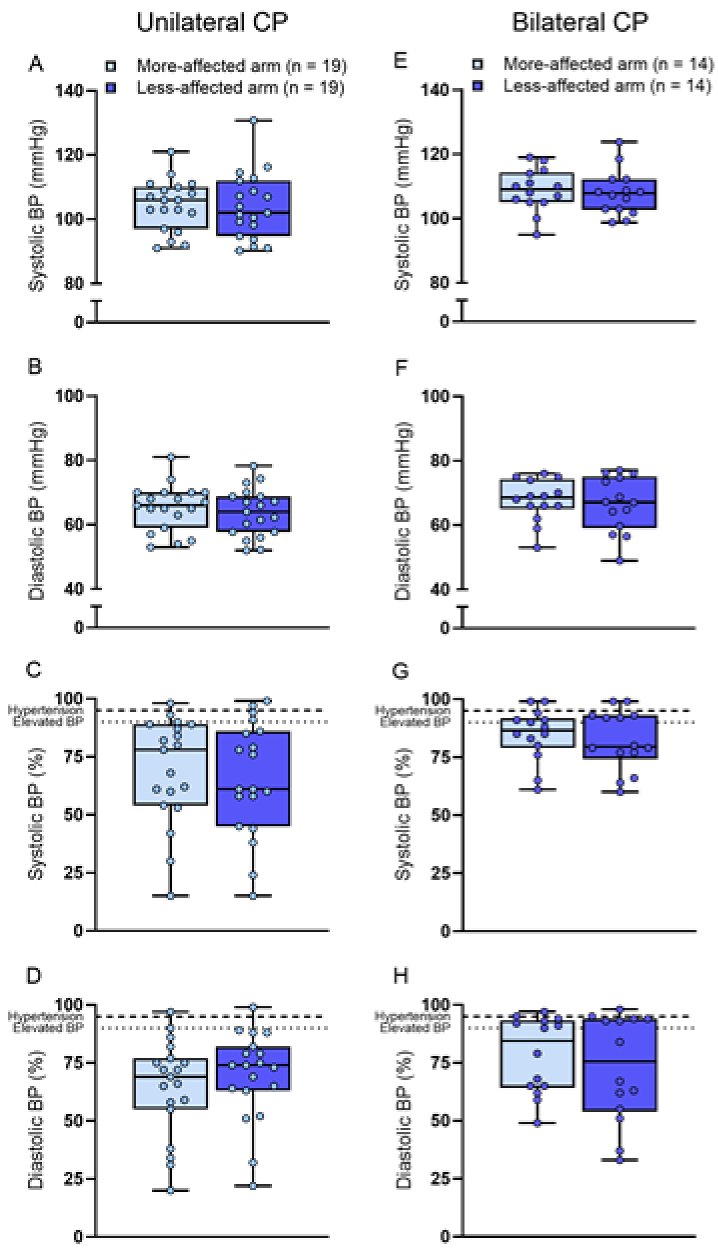

Fig. 12. Comparison of inter-arm blood pressure in children with unilateral and bilateral cerebral palsy (CP). BP: blood pressure; Systolic or diastolic blood pressure more than or equal to the 90th percentile but less than the 95th percentile was classified as elevated blood pressure, and systolic or diastolic blood pressure more than or equal to the 95th percentile was classified as hypertension

The intraclass correlation coefficient (ICC) values for inter-arm blood pressure measurements demonstrated excellent reliability, with an ICC of 0.930 for systolic blood pressure and 0.914 for diastolic blood pressure. These findings indicate a high degree of consistency between measurements obtained from the two arms. Scatterplots in Figure 13 demonstrate strong relationships between blood pressure measurements from each arm in both groups.

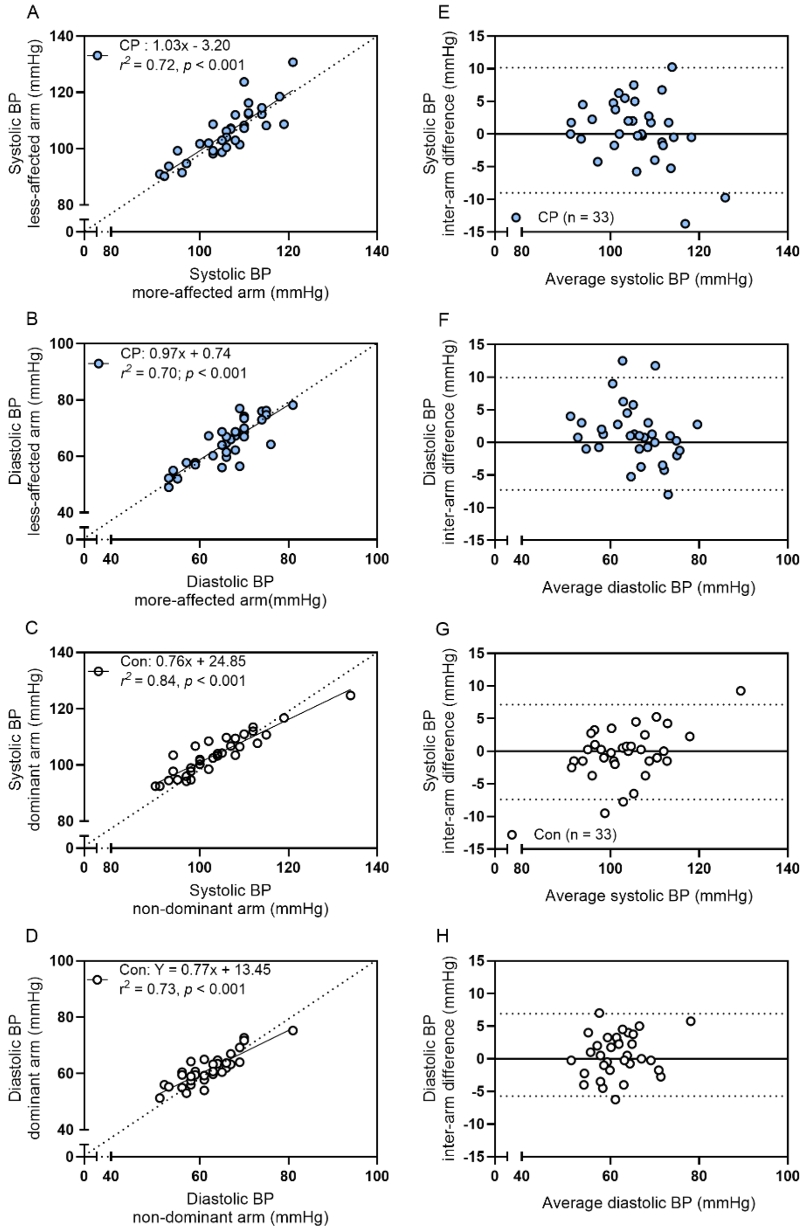

Fig. 13. Scatter plots and Bland-Altman plot for the inter-arm blood pressure measurements in children with cerebral palsy (CP) and typically developing control children (controls). BP: blood pressure; X-axis: average of two measurements; Y-axis: blood pressure measurements from the more-affected arm in children with CP or non-dominant arm in controls - blood pressure measurements from the less-affected arm in children with CP or dominant arm in controls.

When elevated blood pressure and hypertension were classified using blood pressure percentile from the more-affected arm in children with CP and non-dominant arm in controls, 7 children were classified as having elevated blood pressure (21 %), and 4 children were classified as having hypertension (12 %) in children with CP (n = 33), and 3 children were classified as having elevated blood pressure (9 %) and 2 children were classified as having hypertension (6 %) in controls (n = 33). When elevated blood pressure and hypertension were classified using blood pressure percentile from the less-affected arm in children with CP and the dominant arm in controls, 6 (18 %) children were classified as having elevated blood pressure, and 5 (15 %) children were classified as having hypertension in children with CP (n = 33), and 3 (9 %) children were classified as having elevated blood pressure and 1 (3 %) child was classified as having hypertension in controls (n = 33).

Children with CP showed a higher diastolic blood pressure, mean arterial pressure, and diastolic blood pressure percentile on the more-affected arm ($d = 0.663$, 0.549, and 0.726, all $p < 0.05$, respectively) and higher diastolic blood pressure on the less-affected arm ($d = 0.528$, $p = 0.039$) compared to controls.

No significant difference in the prevalence of elevated blood pressure or hypertension was observed between children with CP and controls ($p > 0.05$). In children with unilateral CP, the prevalence of elevated blood pressure or hypertension was similar to that of the controls ($p > 0.05$). In contrast, children with bilateral CP exhibited a higher prevalence of elevated blood pressure or hypertension compared to the controls ($p < 0.05$).

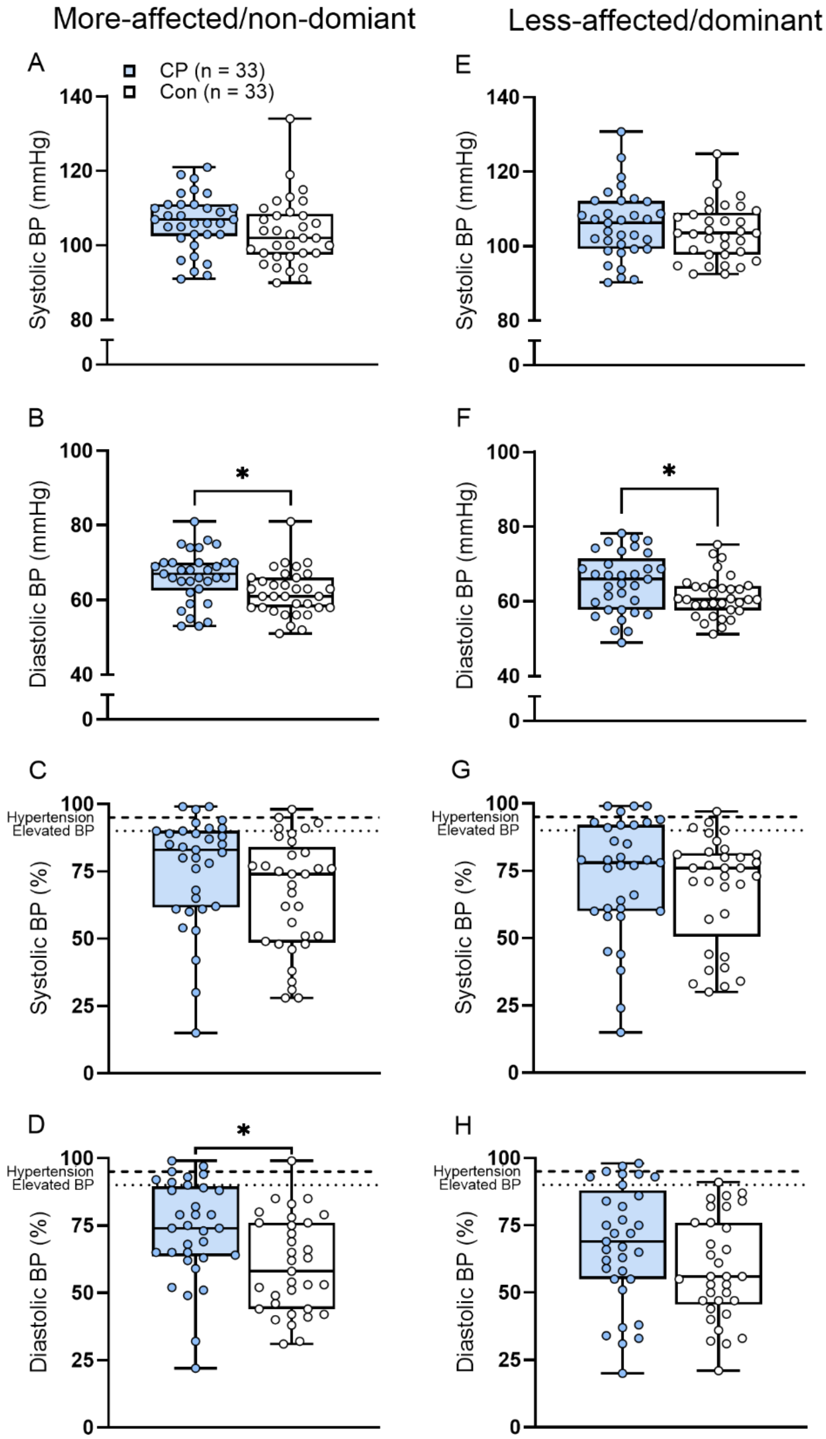

Fig.14. Comparison of blood pressure in children with cerebral palsy (CP) and controls (Con). Systolic or diastolic blood pressure more than or equal to the 90th percentile but less than the 95th percentile was classified as elevated blood pressure, and systolic or diastolic blood pressure more than or equal to the 95th percentile was classified as hypertension. *group difference, $p < 0.05$

5.5 Discussion

To our knowledge, this is the first study to determine whether there are differences in inter-arm blood pressure measurement in children with CP. No significant inter-arm difference in blood pressure measurement in ambulatory children with spastic CP and typically developing controls were observed in the study. This result is inconsistent with the previous literature that observed a higher blood pressure measurement from the spastic arm in patients with hemiplegia[42] and the literature observed a higher prevalence of inter-arm blood pressure difference in post-stroke patients with hemiparesis.[304,305] This result is partially consistent with previous literature that showed no consistent lateralization of blood pressure in young populations.[306] Four children with CP showed a significant inter-arm blood pressure difference (≥ 10 mmHg, 12 %), and no typically developing control showed a significant inter-arm blood pressure difference. This prevalence is lower than the previous literature in young healthy participants (21.4 %).[306] This inconsistency probably results from differences in the methodology applied. In this study, an average of 4 measurements for each arm were used for analysis, while previous literature used the average of 2 measurements.[306] Karagiannis also averaged their blood pressure measurement as done in this study and got a 4.7% prevalence of a significant inter-arm blood pressure difference in middle-aged adults (54 ± 18.28y).[307] More studies on the occurrence of a significant inter-arm blood pressure difference are warranted.

In subgroup analysis, children with unilateral and bilateral CP did not demonstrate inter-arm differences in blood pressure. We had anticipated that the inter-arm difference in blood pressure would be more pronounced in children with unilateral CP, attributed to their hemiplegic symptoms. However, contrary to our expectations, neither group showed a significant inter-arm difference in blood pressure. Interestingly, the four participants who exhibited a significant inter-arm blood pressure difference were children with bilateral CP. This suggests that the severity or type of CP might affect the prevalence of the inter-arm blood pressure difference in children with CP. Given that inter-arm blood pressure differences are positively associated with multiple cardiovascular diseases,[308] this result suggests that children with bilateral CP, who showed great mobility limitation, might have more distinct cardiovascular risk factors. However, further studies with a larger sample size may be needed to confirm this observation.

When elevated blood pressure and hypertension were classified using the blood pressure percentile from each arm, the blood pressure percentile from the more-affected arm classified one less child with CP as hypertension and one more child with CP as elevated blood pressure, while it classified one more control as hypertension in controls, compared to using the blood pressure percentile from the less-affected arm. However, no significant differences in the prevalence of elevated blood pressure or hypertension were observed between children with CP and controls. Moreover, the ICC indicated high consistency between measurements obtained from the two arms. These results suggest that the choice of arm for blood pressure measurement might not affect the detection of elevated blood pressure and hypertension in these children significantly. Clinical practice guidelines for screening and management of high blood pressure in children and adolescents strongly recommend measuring blood pressure in the right arm unless the child has atypical aortic arch anatomy.[40] Considering the local impact of spasticity on blood pressure measurement[42] from the more-affected arm, utilizing the less-affected arm for blood pressure measurement might be more appropriate to identify elevated blood pressure and hypertension in children with CP. However, because it is unclear if potential increases in blood pressure due to spasticity contribute to cardiovascular disease risk, caution should be used when interpreting the results and making appropriate recommendations.

Children with CP exhibited higher blood pressure compared to age- sex- and race-matched controls. This result is consistent with the previous study in our lab.[262] The differences in blood pressure measurement between children with CP and controls were more distinct on the more-affected arm than the less-affected arm, although there was no significant inter-arm difference in blood pressure in either group. This suggests that a local factor could affect the blood pressure in children with CP and could slightly raise the blood pressure in the more-affected arms. It supports the idea that spasticity on the arm might affect blood pressure.[42] However, further studies are required to elucidate it.

It is important to note certain limitations that were not previously mentioned. One of the main limitations of this study is the relatively small sample size, which could impact the generalizability and strength of the results. In the subgroup analysis, unilateral CP encompassed monoplegia and hemiplegia, while bilateral CP included diplegia and quadriplegia. In children with monoplegic and diplegic CP, the measurement of blood pressure in both arms might not be affected by CP. However, further subgroup analyses were constrained due to the limited sample size. Nonetheless, the sample size was sufficient to exhibit the difference in blood pressure between children with CP and the control group. Secondly and lastly, spasticity was not assessed for the upper limb where the blood pressure was measured, as conducted in the study by Lin.[42] Consequently, the effect of spasticity on the blood pressure measurements in children with CP could not be determined. However, no inter-arm difference in blood pressure was observed in any group in this study. Therefore, this limitation may not have impacted the conclusion.

5.6 Conclusion

The results of the study suggest that in most instances, blood pressure measurement taken from the more- or less-affected arms is similar in ambulatory children with spastic CP. However, almost 30 % of children with bilateral CP exhibited a pathologically significant inter-arm difference (>10 mmHg). Thus, assessing blood pressure in both arms may be necessary in children with bilateral CP. In addition, studies determining the impact and relevance of spasticity on arm blood pressure measurement in children with CP are warranted.

CHAPTER 6

CONCLUDING SUMMARY

The overall objective of this dissertation was to assess the vascular health of children with CP. A brief summary of all the three specific aims is below.

Summary of specific aim 1: The aim of the first study was to determine whether the size and hemodynamics of the popliteal artery are compromised in children with CP and whether the expected effects persist after adjusting for lower limb muscle size. The main finding was that children with CP have a smaller popliteal diameter and higher shear rate compared to typically developing controls, which is linked to their small leg muscles. These findings may provide mechanistic insights for elevated cardiovascular disease risk in children with CP.

Future direction: This study only assessed the diameter and shear rate as a vascular health parameter. Future studies should look into the variables related to endothelial function, such as flow-mediated dilation and intima-media thickness. These additional measures will provide a more comprehensive overview of the vascular health in children with CP.

Summary of specific aim 2: The aim of the second study was to examine the relationship between popliteal artery diameter and physical activity and physical function as well as between shear rate and physical activity and function in children with CP. The main findings were popliteal diameter and shear rate were related to lower limb function rather than physical activity in children with CP. 6-minute walk test distance was a significant predictor of vascular measures in CP children. These results suggest that the limited functional capacity of children with CP may suppress popliteal artery growth.

Future direction: This study only assesses physical activity from the monitor worn on the hip due to the lack of appropriate cut-points for physical activity intensity in children with CP. Future studies should look into physical activity from the monitor worn on the ankle, which might provide more related data to the activity limitation of the lower limb in children with CP, with an appropriate cut-point for children with CP.

Summary of specific aim 3: The aim of the third study was to verify the inter-arm differences in blood pressure measurements between the more-affected and less-affected arms in ambulatory children with spastic CP. The main finding was that blood pressure measurement taken from the more- or less-affected arms is similar in ambulatory children with spastic CP. However, almost % of children with bilateral CP (n = 4 of 14) exhibited a pathologically significant inter-arm difference (>10 mmHg). Thus, assessing blood pressure in both arms may be necessary in children with bilateral CP. In addition, studies determining the impact and relevance of spasticity on arm blood pressure measurement in children with CP are warranted.

Future direction: This study only classifies the type of CP into bilateral CP and unilateral CP due to the small sample size. Future studies should look into further subdivided CP types such as monoplegia, hemiplegia, diplegia, triplegia, and quadriplegia. Furthermore, this study did not assess the Modified Ashworth Scale from the upper limb, which would affect the blood pressure measurements. Future studies should determine if there is a relationship between muscle spasticity of the upper limb and inter-arm blood pressure difference in children with CP.

In conclusion, children with CP have a smaller popliteal diameter and higher shear rate compared to typically developing peers. Physical function can alter these adaptations in children with CP. Strategies that can improve vascular health in children with CP are needed.

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