EARLY SIGNS OF CARDIOMETABOLIC DISEASE IN CHILDREN WITH CEREBRAL PALSY

by

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ABSTRACT

PURPOSE: The aim was to determine if biochemical markers of dyslipidemia, pre-diabetes, and hyperleptinemia indicate early signs of cardiometabolic disease (CMD) in children with cerebral palsy (CP), and to determine if the biochemical markers are related to visceral adiposity. **METHODS:** Thirty children with CP and 30 controls matched for age, sex, and race were studied. Biochemical markers were assessed using blood. Visceral adiposity was assessed using dual-energy X-ray absorptiometry. **RESULTS:** Children with CP had significantly higher total cholesterol, low-density lipoprotein cholesterol (LDL-C), non-high-density lipoprotein cholesterol (non-HDL-C), glucose, and leptin, and higher visceral and total adiposity than controls (all $p > 0.05$). Visceral adiposity was significantly and positively related to non-HDL-C, glucose, homeostatic model assessment of Insulin resistance, and leptin. **CONCLUSION:** Biochemical markers of dyslipidemia, pre-diabetes, and hyperleptinemia suggest that children with CP exhibit early signs of CMD, which is related to visceral adiposity.

INDEX WORDS: Cerebral palsy, Pre-diabetes, Visceral adiposity, Physical activity, Cardiometabolic disease, Leptin, Dyslipidemia

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DEDICATION

This document is dedicated to the children who participated in this study, and all children living with disability. You are strong. You are capable. You are awesome!

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CHAPTER 1

INTRODUCTION

Cerebral palsy (CP) is the most common cause of physical disability among children, affecting about 1 in every 313 children in the United States (McGuire, Tian, Yeargin-Allsopp, Dowling, & Christensen, 2019). Children with CP are likely to suffer from poor motor control (Bax, Goldstein, Rosenbaum, Leviton, & Paneth, 2005), low muscle volume and strength (Riad, Haglund-Akerlind, & Miller, 2008; Wiley & Damiano, 1998), and concomitantly lower levels of physical activity than their typically developing peers (Carlon, Taylor, Dodd, & Shields, 2013). As a result, these factors can compound and often result in an accelerated loss of function (Morgan & McGinley, 2014) and health (D. G. Whitney et al., 2018) throughout the lifespan. Along these lines, it has been observed that both adults and children with CP exhibit body composition patterns associated with poor metabolic outcomes, such as an infiltration of fat in the abdomen (Daniel G. Whitney, Singh, Zhang, Miller, & Modlesky, 2018), muscle (Johnson, Miller, Subramanian, & Modlesky, 2009) and bone marrow (D. G. Whitney et al., 2017). Specifically, these body composition patterns have been associated with increased systemic inflammation, dyslipidemia, and insulin resistance in typically developing children and adults (Caprio, Perry, & Kursawe, 2017; Shulman, 2014) and are therefore considered risk factors for the development of cardiometabolic disease (CMD). Adults with CP display both an increased prevalence and accelerated onset of CMD, evidenced by a higher prevalence of hypertension, dyslipidemia, and myocardial infarction in young adults with CP as compared to those without

CP (D. G. Whitney et al., 2018). However, there is a lack of literature investigating CMD risk or its relationship with these body composition patterns in children with CP.

Statement of the Problem

Given that adults with CP show both a higher prevalence and an earlier onset of CMD than those without CP, it is pivotal that risk factors and contributors to CMD progression are identified as early as possible in this population. Studies examining CMD risk among children with CP are lacking. Therefore, it is important to identify feasible target outcomes for preventative care for children with CP.

Specific Aims

Aim 1: The primary aim of this study is to determine if serum biochemical markers indicate increased CMD risk (fasting glucose, homeostatic model assessment of insulin resistance (HOMA-IR), lipids, adiponectin, and leptin) in children with CP compared to typically developing children.

Aim 2: The secondary aim will be to investigate the relationship these biochemical markers have with visceral adiposity in children with CP.

Hypotheses

Hypothesis 1: Children with CP will have higher CMD risk than typically developing children, as indicated by dyslipidemia, pre-diabetes, increased serum leptin, and decreased serum adiponectin.

Hypothesis 2: Non-high-density lipoprotein cholesterol (non-HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, fasting glucose, HOMA-IR, and leptin will be positively associated with visceral adiposity in children with CP.

Hypothesis 3: High-density lipoprotein cholesterol (HDL-C) and serum adiponectin will be negatively associated with visceral adiposity in children with CP.

Significance of the Study

The proposed study is significant because adults with CP are at a higher risk of developing CMD in early adulthood. Although it has been demonstrated that children with CP have a body composition profile consistent with higher CMD risk, it is unclear if this is supported by biochemical markers typically used to assess this risk, such as total cholesterol, LDL-C, HDL-C, non-HDL-C, glucose, and HOMA-IR. Moreover, it is unknown if the elevated CMD risk emerges during childhood. Through a comprehensive assessment of serum biochemical markers and body composition, we will determine if the higher risk of developing CMD in individuals with CP is present during childhood. The results of this study will positively impact the quality of care in this population by both increasing awareness of the accelerated CMD progression faced by individuals with CP and opening the door to more targeted research into prevention of CMD in those with CP throughout the lifespan.

CHAPTER 2

LITERATURE REVIEW

2.1 Cerebral Palsy

Cerebral palsy is the most common cause of physical disability among children, with more than 9,200 diagnoses per year, and is characterized by a non-progressive insult to the brain during cerebral development. Individuals with CP most commonly display poor motor control & posture (Bax et al., 2005), diminished muscle quality (Booth, Cortina-Borja, & Theologis, 2001; Johnson et al., 2009) and function (Riad et al., 2008; Wiley & Damiano, 1998), reduced bone size and density (Modlesky, Kanoff, Johnson, Subramanian, & Miller, 2009; Modlesky, Subramanian, & Miller, 2008), and a concomitant decrease in physical activity levels compared to those without CP (Carlon et al., 2013). Consequently, this population often suffers from substantial functional deficits as well as an increased risk of musculoskeletal injury (McIvor & Samilson, 1966), and comorbidity (Strauss, Cable, & Shavelle, 1999). Perhaps the most concerning aspect of this condition is the nature in which these functional deficits compound on one another to create a compounding cycle that further augments these effects over time.

Cerebral palsy's physical effects are typically classified clinically by affected limb(s), with diplegic indicating affected limbs on both sides of the body, hemiplegic indicating affected limbs on one side of the body, and paraplegia indicating all limbs are affected. Additionally, muscle tone and movement abnormalities can be used for further classification. (i.e., spastic, dyskinetic, etc.)(Bax et al., 2005). The predominant and most validated measure of gross motor

function for those with CP is the Gross Motor Classification System (GMFCS) (R. J. Palisano et al., 2000). The GMFCS uses five classifications (I-V) to indicate increasing severity of functional limitations. Those classified as levels I or II are considered ambulatory, and walk predominantly without the help of any assistive device. While those classified as level III may be capable of walking shorter distances without assistance, they may require an assistive device for longer distances. Additionally, they are capable of traversing stairs with minimal assistance and supervision. Gross Motor Functions Classification System levels IV and V indicate the individual can no longer walk safely without an assistive device, with those in level V confined to a manual wheelchair with a severely limited ability to control their posture against gravity.

2.2 Increased Cardiometabolic Disease in Individuals with Cerebral Palsy

While CP is defined as a non-progressive disorder, overall gross motor function and health in those affected by CP often decreases rapidly throughout the lifespan (Bax et al., 2005). A primary consequence of this exaggerated decline is an increased prevalence of CMD. Cardiometabolic disease encompasses multiple conditions and represents multiple interrelated risk factors, primarily hypertension, dysregulated glucose disposal, dyslipidemia, elevated triglycerides, and increased central adiposity. Together, these factors are also said to represent a "metabolic syndrome" indicative of the development of more distinct conditions such as type 2 diabetes mellitus and cardiovascular disease (Grundy et al., 2005).

Strauss et al. (1999) investigated the contribution of a number of factors to excess mortality in those with CP throughout their lifespan using medical records from 401 individuals with CP and the Client Development Evaluation Report of the state of California (Strauss et al., 1999). The proportion of incidences of mortality in those with CP were compared to normative

values to calculate standardized mortality ratios. Stratified for age, standardized mortality ratios for mortality due to cardiovascular disease ranged from 5.5 in those with CP and aged 0-34 years to 2.2 for those 34 years and above.

A more recent investigation conducted by Heyn et al. (2019) reported that ambulatory adults with CP (GMFCS I-III ; n = 70, average age = 24.4 y) had a 71% higher proportion of metabolic syndrome (as defined by the National Cholesterol Education Program/Adult Treatment Panel III) than was observed by the National Health and Nutrition Examination Survey cohort (Heyn, Tagawa, Pan, Thomas, & Carollo, 2019). Important to note is that this increase was only 21% when using World Health Organization and International Diabetes Federation classifications, which are less dependent on lipid-based factors. Additionally, according to Framingham Heart Study cardiovascular disease 30-year risk factors, CP participants in this study displayed 39.7% and 26.5% greater risk of developing full cardiovascular disease as indicted by body mass index (BMI) and lipid-based factors, respectively. A weakness of the study was that there were no controls without CP included for comparison. However, giving a closer look at the onset of these conditions, Whitney et al. (2018) reported a 1.98 fold increased odds ratio of CMD in young adults (age = 18-30 years) with CP when compared to those without CP (n = 452 with CP, n = 448 without CP) (D. G. Whitney et al., 2018), displaying a perturbingly early onset of CMD in this population.

In summary, while CMD risk is not well documented in children with CP, there is evidence that risk factors for the development of these conditions are frequent in adults with CP, and present earlier in life than those without CP. Hence, additional studies, especially those that include carefully selected controls are needed to determine if signs of CMD progression emerge during childhood.

2.3 Physical Activity in Individuals with Cerebral Palsy

It has been hypothesized that a primary driver of early onset CMD in individuals with CP is low levels of physical activity and a concomitant increase in levels of sedentary activity due to impaired physical function (Verschuren, Peterson, Balemans, & Hurvitz, 2016). This notion is supported by studies reporting diminished levels of physical activity apparent in those with CP early in life (Carlon et al., 2013). It is especially concerning as exposure to mechanical loading and physiological stimulus during childhood and adolescence is very influential in the development of a robust adult musculoskeletal system (Modlesky & Lewis, 2002). Consequentially, loss of function and mobility becomes exaggerated throughout the lifespan at a more rapid rate than observed in the general population (Morgan & McGinley, 2014). This loss is especially pronounced with more rigorous activity (Ryan, Forde, Hussey, & Gormley, 2015), which is more predictive of cardiometabolic health in typically developing children and adolescents (Ekelund et al., 2012).

A study by Bjornsen and colleagues (2007) compared total physical activity and proportion of time spent at different physical activity intensities between 81 ambulatory youth (10-13 years old) with CP and 30 typically developing children. Physical activity was measured by a StepWatch activity monitor (SAM; Orthocare Innovations, Seattle, Washington) calibrated to each participants' walking pattern and worn for one week. They observed significantly lower daily steps, ratio of moderate/low activity, percentage of time spent active, and percentage of time spent at high (moderate to vigorous) activity levels in those with CP ($p < 0.05$). The largest differences were in daily steps (37% lower than controls) and percentage of time spent in high activity levels (73% less than controls) (Bjornson, Belza, Kartin, Logsdon, & McLaughlin, 2007).

Johnson et al. (2009) assessed physical activity levels in children with CP (5 – 11 y) who were unable to ambulate without assistance using accelerometer-based physical activity monitors worn on the non-dominant hip. Compared to typically developing children (n = 12/group), children with CP had 70 % fewer total activity counts, and physical activity accounts were significantly inversely related to intramuscular ($p < 0.01$) and subfascial ($p = 0.03$) adipose tissue concentrations in children with CP ($r = 0.76$, -0.63, respectively)

Furthermore, a study by Ryan et al. (2014) investigated the relationship between activity levels in ambulatory adults with CP (n = 41, ages 18-62) and known CMD risk factors. Physical activity was measured via accelerometer-based monitors for 7 days and compared to sex- and age-matched controls (n = 41). This study also found significantly lower levels of light, moderate, and moderate-to-vigorous activity as well as increased time spent sedentary in those with CP. However, while there were significant relationships observed between moderate physical activity and measurements of abdominal adiposity, systolic blood pressure, and diastolic blood pressure, no significant relationships between any other physical activity measurement, or more direct markers of CMD like glycated hemoglobin, lipids, 25-hydroxyvitamin D, etc. was observed (Ryan, Hensey, McLoughlin, Lyons, & Gormley, 2014). For context, a Dutch study by van der Slot et al. (2013) found no relationship between physical activity and risk factors for CMD in adults with CP while only measuring total physical activity (van der Slot et al., 2013). One important caveat to consider when interpreting these findings is that the measures of physical activity used (pedometers and accelerometry) are purely movement-oriented, and do not fully account for the increased energy expenditure required for individuals with CP to achieve the same activity levels as typically developing children due to biomechanical inefficiencies

(Bell & Davies, 2010). Because of this, physiological effects may not be proportionate to discrepancies in activity levels, especially as they relate to energy expenditure and metabolism.

To summarize, individuals with CP have lower levels of physical activity, higher levels of sedentary activity, and an exaggerated loss of mobility and function with age, which is consistent with an elevated CMD risk.

2.4. Traditional Estimates of Body Composition and Anthropometric-based Markers of Obesity

Knowledge of body composition is important in the assessment of physical fitness, nutritional status, and disease risk. Most traditional methods used to assess body composition are based on a two-component model in which the total body is separated into fat and fat-free components. More sophisticated methods are able to separate the body into more than two components, such as dual-energy X-ray absorptiometry (DXA), which can quantify fat, fat-free soft tissue mass, and bone mineral mass. In addition, although the proportion of fat and fat-free components were previously expressed relative to body weight, it is now more accepted to express them relative to height^2.

Several studies have examined the body composition of children with CP. For example, Stallings et al. (1995) measured patterns of body composition in 136 children with spastic quadriplegic (non-ambulatory) CP (aged 2-12 years) and 39 control children. Importantly, a large proportion of these children with CP (45%) reported severe difficulties relating to oral motor control and feeding. This study reported significantly lower weight, upper arm muscle area, skinfold calculated fat- and fat-free mass, and limb lengths, but higher skinfold thicknesses in children with CP compared to controls and normative data (all $p < 0.01$). Additionally, by

plotting age against fat free mass in both children with CP and controls, the slopes provide evidence that fat-free mass is accrued at a much lower rate with age in children with CP (m = 1.060) compared to controls (m = 2.157).

Oftedal et al. (2017) investigated body composition in a sample of young children with CP (aged 2-5 years) in a wider range of GMFCS categories (I-V). The most affected children (GMFCS V) had significantly lower fat free-mass, while the GMFCS II-IV had significantly higher body fat percentage than the GMFCS I group (all $p < 0.05$). When all groups were pooled together, the average body fat percentage classified the sample as obese, even with an average BMI of only 16.3 (Oftedal et al., 2017). A limitation of this study is its lack of a true control group for direct comparisons.

Prior research indicates that the rate of obesity, as reflected by BMI, in ambulatory children with CP is climbing, but is not significantly greater than that of the general population and may even be slightly lower (Rogozinski et al., 2007). Prevalence of BMI-classified obesity was measured to be even lower still in non-ambulatory children with cerebral palsy. However, there is reason to question the utility of BMI as a description of adiposity or body composition in this population. Whitney et al. (2019) analyzed the body composition of 42 children with CP (n = 24 ambulatory; n = 18 non-ambulatory) with 42 typically developing children matched for age, sex and race. While the total sample of children with cerebral palsy compared to typically developing children had no difference in BMI ($p = 0.767$) or total body fat ($p = 0.102$), the children with cerebral palsy had significantly higher body fat percentage ($p = 0.001$), fat mass index (FMI) ($p = 0.003$), and significantly lower fat-free mass ($p = 0.001$) and fat-free mass index (FFMI) ($p = 0.005$).(D. G. Whitney, Miller, Pohlig, & Modlesky, 2019).

Similarly, a previous study by Kuperminc and colleagues investigated the accuracy of single anthropometric measures of body composition (body mass index, mid-upper arm circumference, triceps skinfold, and mid-upper arm fat area) in 58 children with CP, GMFCS III-V. None of the single anthropometrics accurately predicted body fat percentage (measured by DXA, weighted kappa ranging from 0.04-0.09) in these children. They were slightly more accurate in typically developing children, according to National Health and Nutrition Examination Survey data (weights kappa ranging from 0.44 to 0.52). All measures tended to underestimate body fat percentage in both groups. A limitation of this study is that height was estimated using knee height in the children with CP due to issues with posture, fixed contractures, involuntary spasms, etc. (Kuperminc et al., 2010).

These results provide evidence that diminished lean body mass as a result of the musculoskeletal deficiencies observed in those with cerebral palsy severely limits the ability of BMI to accurately describe body composition as it pertains to adiposity. Consideration of these differences in body composition are of significant clinical importance as low levels of fat-free mass, which includes lean body mass, are not only detrimental to functional ability, but also results in decreased resting metabolic rate (Hopkins et al., 2016) which could have negative implications on overall metabolic health.

2.5 Ectopic and Visceral Fat

Although disease risk is related the degree of fat in the total body, there is evidence that its' relationship is stronger with fat in specific regions of the body, such as within muscle, bone marrow, and the visceral cavity. Increased accumulation of ectopic fat has adverse metabolic effects that increase the risk of developing CMD in adults (Shulman, 2014; Tchernof & Despres,

2013) and obese children (Caprio et al., 2017). The relationship between increased ectopic fat accumulation and CMD risk is independent of total body fat (Cali & Caprio, 2009; Taksali et al., 2008). The prevailing explanation of how and why ectopic fat, as opposed to subcutaneous adipose tissue, accumulates and elicits its deleterious metabolic effects in both children and adults is the "adipose tissue expandability" hypothesis (Caprio, Pierpont, & Kursawe, 2018; Gray & Vidal-Puig, 2007). In theory, each individual's subcutaneous adipose tissue has a set capacity to expand and store lipids, which is likely limited by adipocyte hypertrophy as adipocyte number seems to be less malleable. Thus, when the storage capacity is exceeded, lipids begin to accumulate ectopically and infiltrate skeletal muscle, bone marrow, the liver, pancreas, and kidneys and create a potentially lipotoxic and inflammatory environment. While the relationship between ectopic fat and CMD risk is likely mediated by numerous interrelated conditions, systemic and unresolved inflammation lays the foundation of ectopic fat's adverse metabolic effects (Kranendonk et al., 2015). Inflammatory cytokines and ceramides excreted by ectopic fat depots have been linked to increased risk of developing insulin resistance, dyslipidemia, and therefore CMD (Shulman, 2014).

While this "overflow" of lipids into ectopic fat is believed to begin in skeletal muscle, leading to peripheral insulin resistance, the pathogenesis eventually leads to an accruement of adipose tissue elsewhere, including in the viscera. This tissue located inside the visceral cavity and can surround key organs involved in metabolic homeostasis. Evidence of increased pro-inflammatory secretions of visceral adipose tissue was provided by an elegant study by Fontana et al. (2007) that compared cytokine levels at the portal vein, which drains visceral fat, and the radial artery in 25 severely obese adults. They observed a 50% greater interleukin-6 concentration in the portal vein compared to the radial artery, which correlated moderately with

systemic C-reactive protein concentrations ($r = 0.544$, $p = 0.005$) (Fontana, Eagon, Trujillo, Scherer, & Klein, 2007). The observation is important because interleukin-6 is an inflammatory adipokine associated with insulin resistance and diabetes. Additionally, there is evidence that this inflammatory milieu associated with visceral adipose tissue accumulation can present early in life. Visceral adiposity has been shown to display increased inflammatory gene expression (Tam et al., 2011), and correlate with serum inflammatory cytokines (Gaines et al., 2016) in obese typically developing children.

Evidence indicates that children with CP display an increased accumulation of ectopic fat compared to typically developing children. Johnson et al. (2009) compared intermuscular adipose tissue infiltration at mid-thigh between 12 children with quadriplegic CP (Aged 5-14 years, GMFCS III-V) and 12 typically developing children matched for age, sex, and pubertal development. Children with CP in this study had a 2.3-fold greater intermuscular adipose tissue cross-sectional area ($p = 0.01$), with no difference in total or subcutaneous adipose tissue ($p > 0.05$), and a 42% higher adipose tissue fraction within soft tissue than the typically developing children ($p < 0.001$). This study also reported 51% lower muscle cross-sectional area in the CP group ($p < 0.001$), consistent with other studies regarding body composition in children with CP (Azcue, Zello, Levy, & Pencharz, 1996; Chad et al., 2000; D. G. Whitney et al., 2019). Importantly, this study also measured physical activity with accelerometer-based monitors and reported that both the ratio of intermuscular adipose tissue cross-sectional area /muscle cross-sectional area and subfascial adipose tissue cross-sectional area /muscle were significantly inversely related to total physical activity counts in children with CP ($r = -0.76$, $p < 0.01$; $r = -0.63$, $p = 0.03$), but not in typically developing children (Johnson et al., 2009). A more recent study by D'Souza and colleagues (2020) measured intramuscular fat infiltration in the lower leg

(gastrocnemius and tibialis anterior) in ambulatory children with unilateral CP (n = 20, aged 5-18 years, GMFCS I-II) and typically developing children (n = 20) of the same age range. In this group of mildly affected children with CP, intramuscular adipose tissue fraction was significantly higher in both muscles than in controls (gastrocnemius = 4.7%, tibialis anterior = 4.6%) for both the affected (gastrocnemius = 11.4%, tibialis anterior = 10.0%) and non-affected limbs (gastrocnemius = 6.9%, tibialis anterior = 5.9%) (all $p < 0.05$) (D'Souza, Bolsterlee, Lancaster, & Herbert, 2020).

Additionally, while less studied, there is emerging evidence that children with CP also exhibit increased visceral adiposity. A recent study by Whitney et al. (2018) compared total body, trunk, and visceral fat and fat-free mass estimates from DXA of ambulatory (GMFCS I-II) children with CP and sex-, age-, and race-matched typically developing children (n = 18/group) using DXA. Total body fat mass, fat-free mass, fat-mass index (FMI), and fat-free mass index (FFMI), as well as trunk fat mass, fat-free mass, and FFMI, or abdomen fat mass, visceral fat mass, and subcutaneous fat mass were not different between groups ($p > 0.05$). However, children with CP displayed a significantly greater trunk, abdomen, and visceral fat FMI (all $p < 0.05$), which demonstrated a greater degree of central adiposity (Daniel G. Whitney et al., 2018). These findings are consistent with those in adults with CP (GMFCS I-V, average age = 39.4). Using computed tomography (CT), Peterson et al. (2015) observed a significantly greater amount and proportion of visceral fat when compared to controls, but no group difference in body mass, BMI, or subcutaneous fat (Peterson, Zhang, Haapala, Wang, & Hurvitz, 2015).

Clinical measurements of central adiposity, such as waist-to-hip ratio (WHR) and waist circumference, have been widely used to estimate visceral adipose tissue accumulation since the early 1980's. They are convenient and show moderate relationships with CMD risk (Lapidus et

al., 1984; Ohlson et al., 1985). However, they do not reliably estimate actual visceral adipose tissue accumulation, as measured by CT (Despres, Lemieux, & Prud'homme, 2001; Lemieux et al., 2000).

Computed tomography has been shown to accurately discriminate between adipose tissue depots (Sjostrom, Kvist, Cederblad, & Tylen, 1986) and is considered the gold standard of visceral adipose tissue measurement. However, it is not without limitation. The radiation emitted by CT scan makes them less than practical, especially in children or when multiple scans are needed. Magnetic resonance imaging (MRI) is also often used to measure visceral adipose tissue accumulation, as it does not emit radiation and has also been shown to accurately measure adipose tissue (Fowler, Fuller, Glasbey, Cameron, & Foster, 1992) and shows results consistent with those from CT scanning (Seidell, Bakker, & van der Kooy, 1990). Unfortunately, the participant must also lay motionless and the time it takes to obtain an accurate MRI scan is even longer than that of CT scans (often taking upwards of 15 minutes) which makes it very difficult to complete in many populations.

Dual-energy X-ray absorptiometry has more recently emerged as a viable option to measure body composition and visceral fat in those where CT or MRI is not suitable. A study by Micklesfield and colleagues (2012) compared both visceral adipose tissue measurements from DXA, and measurements from a CT scan analyzed by a clinician, to those from a CT scan analyzed by an expert. The DXA measurement correlated with the expert reading (both $r = 0.93$, $p < 0.001$) as well as the clinician's reading (Micklesfield, Goedecke, Punyanitya, Wilson, & Kelly, 2012). Importantly, a DXA instrument emits minimal radiation, and a whole body scan can be completed in 10 minutes or less, making it a very feasible option for children and special populations. Another study published in the same year (Kaul et al., 2012), showed even more

favorable results when comparing DXA visceral adipose tissue measurements to those of a CT scan ($r = 0.974$ for men; $r = 0.979$ for women; both $p < 0.001$). Dual x-ray absorptiometry measurement of visceral adipose was also validated against MRI in a sample of 237 Middle Eastern men (n =130) and women (n = 107) ($r = 0.95$, $p < 0.001$)(Mohammad et al., 2017). DXA measurements showed a mean bias of overestimation of about 7% in both men and women, with slightly less accuracy observed in those with higher levels of visceral adipose tissue.

There is also evidence the DXA estimations of visceral adipose tissue are related to CMD risk factors in children (Bosch et al., 2015). A study by Bosch and colleagues showed a lower consistency between DXA and CT readings of visceral adipose tissue than prior studies in a sample of boys and girls of a wide range of ages (6-18 years) ($r = 0.626$). However, both methods showed significant relationships with dyslipidemia, with both being significantly inversely associated with HDL cholesterol ($r = -0.286$, $p = 0.001$) and positively associated with serum triglycerides, LDL-C (r range = 0.158 to 0.445, all $p < 0.03$), and insulin sensitivity measured by euglycemic clamp ($r = 0.424$, $p < 0.001$ for DXA; $r = 0.393$, $p = 0.03$ for CT). Interestingly, DXA measurements showed a statistically significant relationship with the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) while CT did not ($p = 0.02$ for DXA; $p = 0.33$ for CT), even when CT displayed a slightly stronger correlation ($r = 0.260$ for DXA, $r = 0.277$ for CT).

2.6 Glucose, Insulin, and Insulin Resistance

As discussed above, the release of ceramides and cytokines, including IL-6 and CRP, from ectopic fat is associated with increased systemic inflammation. This unresolved inflammatory state is tightly linked to the onset and attenuation of unfavorable metabolic

consequences, primarily driven by the development of insulin resistance (Shoelson, Lee, & Goldfine, 2006). Insulin resistance is central to the pathogenesis of CMD, as it is not only indicative of the development of type 2 diabetes, but it has also been associated with atherosclerosis and cardiovascular disease (Haffner, 1999; Haffner et al., 1999). Inflammatory molecules, like ceramides and certain cytokines released by ectopic fat, can produce a lipotoxic environment around key organs responsible for metabolic homeostasis (skeletal muscle, liver, etc.). This in turn induces and/or attenuates hepatic and peripheral insulin resistance by a number of proposed mechanisms including decreased expression of glucose transporters, which bring glucose into the cell for oxidation, and the disruption of insulin receptor substrate expression and/or signaling (Shulman, 2014; Tilg & Moschen, 2008).

Current literature documenting insulin resistance or its relationship with visceral or ectopic adiposity, in children with cerebral palsy is lacking. However, a study by Pingel (2019) did provide some insight into the inflammatory state of this population. This study compared circulating levels of transforming growth factor beta 1 (TGFβ-1), CRP, and IL-6 in 10 between adults with CP (GMFCS I-IV), 10 control adults, and 14 children with CP (GMFCS I-IV). There were no significant differences measured between adult groups for any outcomes. However, children with CP showed significantly higher levels of TGFβ-1 and CRP than normative values of children without CP, and of adults both with and without CP. Our ability to draw any significant conclusions from this study are severely limited by the lack of a children's control group, and by the very small sample size. This study did not involve any measurement of insulin resistance (Pingel et al., 2019).

Previous studies outline a high prevalence of metabolic syndrome and CMD in adults with CP (Heyn et al., 2019; D. G. Whitney et al., 2018), to which insulin resistance is believe to

be central to, however, to our knowledge there are no studies comparing insulin sensitivity and glucose disposal in CP to controls. nevertheless, inferences can be made using research on those with spinal cord injury (SCI). Duckworth et al. (1980 & 1983) compared glucose tolerance and insulin sensitivity between those with SCI and controls, and further investigated factors related to these outcomes in those with SCI. Participants with SCI were split into glucose tolerant and intolerant groups using an oral glucose tolerance test (OGTT) and classification standards determined by the National Diabetes Data Group. Twenty-three of 42 (55%) individuals with SCI were considered diabetic by these standards. Both the groups classified as tolerant and intolerant with SCI displayed impaired glucose disposal when compared to controls, evidenced by higher sustained elevations in blood glucose than controls at all time points after the OGTT ($p < 0.05$, $p < 0.01$ respectively) (Duckworth et al., 1980). Duckworth and colleagues later found that glucose intolerance in those with SCI is marked by hyperinsulinemia upon an OGTT, and 40% of those with SCI displayed resting serum insulin above normative values, providing evidence for peripheral insulin resistance (Duckworth, Jallepalli, & Solomon, 1983).

This glucose intolerance and peripheral insulin resistance has also been linked to ectopic adiposity in those with SCI. Elder et al (2004) compared a sample of 12 individuals with SCI to 9 height-, weight-, and BMI matched controls. After an OGTT, they not only found that those with SCI showed inhibited glucose disposal and increased insulin secretion at all time points (60, 90, 120 minutes) when compared to controls (all glucose $p < 0.01$), all insulin $p < 0.05$), but that thigh intramuscular fat content was significantly related to both glucose and insulin at all time points as well (r^2 range = 0.71-0.40). Given the similarities between those with SCI and those with CP as it pertains to ectopic adiposity and physical function, it is likely that individuals with

CP show a higher prevalence of impaired glucose disposal and insulin resistance than their typically developing peers.

2.7 Dyslipidemia

Dyslipidemia is a broad term that refers to an imbalance, or abnormal amounts, of blood lipids. These blood lipids include triglycerides and cholesterol, where total cholesterol is also further classified depending on the lipoprotein that carries it. The lipoproteins are most commonly classified as HDL-C, LDL-C, and very low-density lipoprotein cholesterol (VLDL-C). Additionally, non-HDL-C, which includes both LDL-C and VLDL-C, has recently gained traction as a marker of atherosclerotic and CMD risk (Packard & Saito, 2004; Ridker, Rifai, Cook, Bradwin, & Buring, 2005; Zhang et al., 2016). Much of its utility is due to its ability to estimate lipoprotein carriers of apoliprotein B, which is directly indicated in the pathogenesis of atherosclerosis, as it both provides a binding site for lipoproteins to accumulate on the endothelium where they become oxidized, leading to atherogenic inflammation (Olofsson & Boren, 2005).

Dyslipidemia has been well documented in adults with CP. Heyn *et al.* (2019) studied 70 ambulatory adults with CP (aged 18-49 years, average of 24.4 ± 5.4 years), and observed that 21.4% of the cohort met the WHO criteria for dyslipidemia, while 40% of the cohort met criteria set by the NCEP ATP III and the IDF (Heyn et al., 2019). Another previously mentioned study by Whitney *et al.* (2018) was able to compare proportions of those with hyperlipidemia (total cholesterol >240 mg/dL and/or triglyceride >200 mg/dL) in a large sample of young adults (aged 20-30 years) both with and without CP, finding that while rates of hyperlipidemia were lower

than other comorbidities like hypertension, 3% of those with CP presented with it compared to only 1.1% of those without CP.

While dyslipidemia has not been well documented in children with CP, there has been investigation into dyslipidemia in those with Down syndrome. Magge et al. (2009) showed higher LDL-C, triglycerides, and non-HDL-C, with lower HDL-C in youth with Down syndrome (n = 150) compared to youth without Down syndrome (n = 103) between the ages of 10 and 20 years old (all $p < 0.001$) (Magge, Zemel, Pipan, Gidding, & Kelly, 2019).

2.8 Adipokines

Adipose tissue is not only responsible for energy-storage, but displays characteristics of a potent endocrine organ (Kershaw & Flier, 2004). Adipose tissue has the ability to exert both autocrine and paracrine effects on whole body energy homeostasis via excretion of bioactive compounds termed adipokines. These adipokines include growth factors, including a number of cytokines, and hormones, namely adiponectin and leptin. These compounds have important roles in the maintenance of whole body metabolism, and have the potential to become dysregulated in a number of pathological states, including obesity (Leal Vde & Mafra, 2013). Importantly, this dysregulation is linked to both the etiology and progression of CMD (Scheja & Heeren, 2019).

Increased expression of adiponectin has been reported to reverse insulin resistance (Yamauchi et al., 2001), ectopic fat accumulation (Xu et al., 2003) and dyslipidemia (Xu, Yin, Wong, Chan, & Lam, 2004) in animal models. This is believed to be due to a number of mechanisms, including the upregulating ceramidase expression thus exerting an anti-inflammatory effect by degrading ceramidase (Holland et al., 2017), and increased glucose & fatty acid uptake into skeletal muscle along with decreased hepatic glucose production (Yanai &

Yoshida, 2019). Additionally, there also evidence that adiponectin exerts direct effects on endothelium and smooth muscle, and therefor may protect against vascular dysfunction directly (Goldstein & Scalia, 2004). In human adults, high levels of adiponectin are associated with a lower risk of developing type 2 diabetes (Li, Shin, Ding, & van Dam, 2009) as well as lower incidence of acute coronary syndromes (Pischon & Rimm, 2007) and myocardial infarction (Pischon et al., 2004). Following, low levels of adiponectin are correlated with ectopic adiposity, insulin resistance, dyslipidemia, and atherosclerosis, and are considered a risk factor for CMD (Matsuzawa, Funahashi, Kihara, & Shimomura, 2004).

Adiponectin's relationship with ectopic adiposity is present not only in adults, but in children and adolescents as well. A study of 53 obese and 30 non-obese, age matched Japanese children reported both significantly lower serum adiponectin levels in the obese children ($p < 0.001$), and a significant negative correlation between serum adiponectin levels and visceral adiposity ($r = -0.531$, $p < 0.001$) (Asayama et al., 2003). Importantly, as these children participated in a weight loss intervention, serum adiponectin levels rose in conjunction with visceral fat loss. These correlations remained significant even when corrected for body fat percentage. Similarly, low serum adiponectin levels have been observed to be a strongly related to intramuscular lipid infiltration in adolescents (Weiss et al., 2003). However, it is important to note that while studies on children and adolescents have shown protective effects of high adiponectin levels on CMD risk (Punthakee et al., 2006), there is inconsistency in the relationship between low adiponectin and CMD risk factors (Stefan et al., 2002).

Adiponectin levels have not been thoroughly investigated in children with cerebral palsy. To our knowledge, a single study conducted by Osateerakun et al. (2019) reported that serum adiponectin levels are weakly, but significantly, negatively correlated to BMI ($r = -0.25$, $p =$

0.03), but not significantly related to GMFCS in a sample of 72 children with cerebral palsy (Osateerakun, Weerasopone, Amarase, Honsawek, & Limpaphayom, 2019). Little is known regarding adiponectin levels in children with cerebral palsy compared to typically developing children, but given adiponectin's inverse relationship with ectopic adiposity, it is possible that children with CP display lower serum adiponectin levels than their typically developing peers.

Investigations into a similar population, children with Down syndrome, have shown mixed results. While two studies measured adiponectin levels, only one found significantly lower levels in those with Down syndrome compared to controls. This study on Egyptian children by Yahia et al. (2021) involved 3 groups, 50 obese children with down syndrome, 50 obese healthy children, and 50 non-obese health children. Not only did obese children with down syndrome show decreased adiponectin levels- but also increased triglycerides, insulin and HOMA-IR along with significantly lower HDL-C values compared to obese-control (all $p < 0.05$) compared to healthy obese children. Additionally, adiponectin was more strongly related to metabolic syndrome in those with Down syndrome (AUC = 0.808) than in obese controls (AUC = 0.674). An earlier study by Tenneti and colleagues (2017) observed lower adiponectin levels in non-obese children (aged 2-12 years) with down syndrome (n = 21) than in matched controls (n = 21), but the difference was not statistically significant ($p = 0.21$). This study also did not find increased risk of insulin resistance or dyslipidemia between groups- however, those with Down syndrome did show significant increases in leptin ($p < 0.01$).

Leptin is a hormone secreted by primarily by adipose tissue that has receptors at the hypothalamus and is responsible for energy homeostasis. Under normal physiological conditions, increases in circulating leptin inhibits appetite and upregulates thermogenesis, fatty acid oxidation, and glucose uptake into skeletal muscle (Ahima & Flier, 2000). Paradoxically, leptin

levels correlate with increased body fatness. This relationship is due to the development of "leptin resistance", similar to the development of insulin resistance (Munzberg, Bjornholm, Bates, & Myers, 2005). This resistance is evidenced to be rooted in both a desensitization of the leptin receptor (ObR), and an inability of leptin to cross the blood-brain barrier, resulting in a dampened signal from the hypothalamus and a decreased ability to regulate energy homeostasis (Caro et al., 1996; R. L. Martin, Perez, He, Dawson, & Millard, 2000). In addition to loss of leptin function associated with central leptin resistance, these pathological increases in circulating leptin are inflammatory and believed to mediate the relationship between increased leptin levels and chronic inflammation and insulin resistance (S. S. Martin, Qasim, & Reilly, 2008). Leptin is also implicated in regulation of bone remodeling via its actions on the immune system but these mechanisms are outside of the scope of this review (Abella et al., 2017; Amling, Takeda, & Karsenty, 2000).

A study by Osateerakun et al. (2019) found higher serum leptin concentrations with increased BMI in children with CP both when participants were split into BMI groups (thin, normal, & overweight), and observed greater leptin levels in the overweight group. Furthermore, leptin was correlated with BMI directly ($r = 0.52$, $p < 0.001$) (Osateerakun et al., 2019). Yakut et al. (2006) measured leptin levels in children with CP (n = 40) and controls (n = 18). The CP included a wide range of clinical presentations (hemiparesis n = 5, quadriparesis n = 28, dyplegia n = 3, mixed n = 4) of the disorder, but the authors did not report GMFCS levels. The CP group was further split up into two groups, decreased- (DSF) and non-decreased subcutaneous fat (non-DSF) (both n = 20), dependent on subcutaneous fat measurement by tricep skinfold thickness. Importantly, the non-DSF group had skinfold thickness measures that were similar to the control group, while the DSF group had significantly lower measurements. Non-DSF children with CP

showed significantly higher leptin levels than controls ($p < 0.01$), while the DSF had significantly lower levels ($p < 0.05$). The CP group as a whole had significantly lower subcutaneous fat on average, along with higher average leptin levels- however this difference was not significant ($p > 0.05$).

Together, these studies provide evidence of elevated leptin levels in children with CP, even at similar body fat levels as typically developing children, which could be indicative of leptin resistance. Hyperleptinemia, leptin resistance, and adipokine dysregulation as a whole has been implicated in increased ectopic fat deposition (Boutari & Mantzoros, 2020; Gabriel et al., 2021; Perseghin et al., 2007) and may influence the increased ectopic adiposity observed in children with CP.

CHAPTER 3

EARLY SIGNS OF CARDIOMETABOLIC DISEASE IN CHILDREN WITH CEREBRAL PALSY[1]

[1]Batson, W.T., Kindler, J., Pollock, N., and C. M. Modlesky. To be submitted to *JAMA Pediatrics.*

ABSTRACT

PURPOSE: Adults with cerebral palsy (CP) display both an increased frequency and an accelerated progression of cardiometabolic disease (CMD) compared to those without CP. The aim of this study is to determine if ambulatory children with spastic CP exhibit early signs of CMD, as measured by biochemical markers, compared to typically developing children, and to examine the relationship of these markers with visceral adiposity. **METHODS:** Thirty children with CP and 30 typically developing children matched for age, sex, and race were studied. Biochemical markers of CMD risk were assessed using fasting blood samples, and body composition was assessed using dual-energy X-ray absorptiometry. **RESULTS:** There were no group differences in age, height, weight, or BMI ($p > 0.05$). Compared to typically developing children, children with CP had a lower height percentile and physical activity ($p < 0.05$). Children with CP had significantly higher total cholesterol, low-density lipoprotein cholesterol (LDL-C), non-high-density lipoprotein cholesterol (non-HDL-C), glucose, and leptin concentrations than controls (all $p < 0.05$). In addition, children with CP had higher fat mass index, and visceral fat index (visceral fat mass/height^2) (all $p < 0.05$). Visceral fat mass was also higher in children with CP, though the difference was marginally insignificant ($p = 0.076$). Visceral fat mass and VFMI were significantly related to HOMA-IR in children with CP ($r =$ 0.811 and 0.698, respectively, $p < 0.001$), but not in controls ($r = 0.256$ and 0.125, respectively, $p > 0.15$). Visceral fat mass and VFMI were significantly related to leptin in the combined sample of both groups (r range 0.714 and 0.611, respectively, $p < 0.001$). **CONCLUSION:** Children with CP demonstrate early signs of CMD including dyslipidemia, pre-diabetes, and hyperleptinemia, and this is related to increased visceral adiposity.

Introduction

Cerebral palsy (CP) is the most common cause of physical disability among children, affecting about 1 in every 313 children in the United States (McGuire et al., 2019). It is the result of a non-progressive insult to the brain during cerebral development, and is characterized by deficits in motor control, posture, and muscle tone (Bax et al., 2005). Consequentially, those with CP often display decreased muscle quality (Booth et al., 2001; Johnson et al., 2009) and function (Riad et al., 2008; Wiley & Damiano, 1998), smaller and more fragile bones (Modlesky et al., 2009) and up to 70% lower levels of physical activity (Johnson et al., 2009) than those without CP. While the condition itself is defined as non-progressive, these deficits are intertwined and compound on one another throughout the lifespan. This often manifests as a significant decline in physical function through childhood and adolescence and into adulthood (Hanna et al., 2009). Concomitantly, those with CP exhibit both a higher prevalence (Heyn et al., 2019; Strauss et al., 1999) and an earlier onset (Johnson et al., 2009; D. G. Whitney et al., 2018) of cardiometabolic disease (CMD). While there is literature documenting the pervasiveness of risk factors for CMD (i.e., dyslipidemia, impaired glucose disposal, altered body composition) in adults with CP (Heyn et al., 2019; D. G. Whitney et al., 2018), less is known about the prevalence of these risk factors in childhood or adolescence. Due to the progressive nature of these functional and health impairments, it is paramount that the presence of these risk factors are investigated and addressed early in life.

Increased accumulation of ectopic fat, which is strongly associated with the development of CMD (Shulman, 2014), has been observed intramuscularly (D'Souza et al., 2020; Johnson et al., 2009; D. G. Whitney et al., 2017) and in the bone marrow (D. G. Whitney et al., 2017) of children with CP. More recently, increased visceral adiposity has been reported in

nonambulatory children with CP, independent of BMI or body fat percentage (Daniel G. Whitney et al., 2018). Visceral adiposity is associated with increased inflammatory and decreased anti-inflammatory gene expression (Kursawe et al., 2016; Kursawe et al., 2010), as well as a serum biochemical marker profile reflective of CMD progression in obese typically developing children and adolescents (Kursawe et al., 2016; Taksali et al., 2008). In addition, increased visceral adiposity has been included as a component of the metabolic syndrome (Eckel, Alberti, Grundy, & Zimmet, 2010).

Despite the elevated levels of visceral adiposity, decreased physical activity, and increased prevalence of CMD later in life in individuals with CP, the state of the CMD biochemical marker profile in individuals with CP, irrespective of age, remains unclear. Studies investigating these markers in direct comparison to typically developing children, are lacking. Moreover, their relationship with visceral adiposity and physical activity in children with CP is unknown. Furthermore, a large proportion of the research involving visceral adiposity is focused on obese individuals. Children with CP display a novel phenotype of high visceral adiposity and low lean body mass, but total body fat levels comparable to those of non-obese typically developing children (D. G. Whitney et al., 2019; Daniel G. Whitney et al., 2018). Investigating the connection between visceral adipose tissue and other markers of CMD risk could provide insight into the unique contribution of visceral adiposity to CMD risk in children with CP, as well as other non-obese (as classified by BMI) populations with a similar phenotype.

The primary aim of this study was to determine whether children with CP display early signs of CMD development, as reflected by levels of biochemical markers, when compared to their typically developing peers. The secondary aim was to determine the relationship of these markers with measures of visceral adiposity.

Methods

Participants

Ambulatory children with spastic CP and 5 to 11 years of age (n = 41) were recruited from the Children's Healthcare of Atlanta, the AI duPont Hospital for Children, public schools throughout the state of Georgia, and pediatric rehabilitation offices throughout the southeast region of the United States as part of different studies involving children with CP. Typically developing children who were similar in age, sex, and race to the children with CP and not participating in high-level sports (n = 32) were recruited from Athens and Atlanta Georgia, the Newark, DE area and surrounding communities. Recruitment was conducted through the use of flyers, postcards, and word of mouth. Children with CP and typically developing children that were the same age (± 1.5 y), sex (male/female), and race (Asian/Black/White) were matched and included in the study

The study was approved by the University of Georgia Institutional Review Board. Before participation in the study, parents or guardians provided informed consent and children provided assent, if able. Exclusion criteria included prior fracture in both femurs or tibias, currently taking bisphosphonates, unable to stand independently, orthopedic surgery within the last six months, children with pure athetoid CP, baclofen pump in the abdomen, and botulinum toxin treatment within the last year.

Anthropometrics

Height and weight were measured while the child was in a t-shirt and shorts. Height was measured to the nearest 0.1 cm using a stadiometer (Seca 217; Seca GmbH & Co. KG.,

Hamburg, Germany). Weight was measured to the nearest 0.2 kg using a digital scale (Detecto, 6550, Cardinal Scale, Webb City, MO). Body mass index (BMI) was calculated based on height and weight. Normative data published by the Centers for Disease Control and Prevention (Kuczmarski et al., 2000) were used to determine age- and sex-based percentiles of height, body mass, and BMI.

Gross Motor Function

Gross motor function was assessed by a trained healthcare professional using the GMFCS. The classification system ranges from I to V. A classification of GMFCS I and II are independently ambulatory, but have a reduced gait speed; GMFCS III achieve mobility through the use of assistive walking devices; and GMFCS IV and V achieve mobility through the use of a wheelchair (R. Palisano et al., 1997). This study included children classified as GMFCS I or II.

Body Composition

Body composition was assessed using whole body dual-energy X-ray absorptiometry (DXA) scans acquired using standard imaging and positioning protocols (Whole Body Analysis; Hologic Inc., Bedford, MA). All images were analyzed using APEX software version 5.6.0.5. To limit motion of children with CP who were unable to remain still without assistance, a modified version of the BodyFIX (Medical Intelligence Inc, Schwabmunchen, Germany) procedure was used to secure them from the waist down, as previously described (Modlesky et al., 2010). The modified BodyFIX procedure has no effect on body composition estimates from DXA in children (Rawal, Miller, & Modlesky, 2011). After completing the scan, total body (excluding the head) fat mass and fat-free mass were determined using standard analysis procedures. Total

body (excluding the head) fat mass index (FMI) and fat-free mass index (FFMI) were determined by dividing tissue mass (kg) by height (m) squared as follows:

$$FMI = \text{fat mass (kg)}/\text{height (m)}^2$$

$$FFMI = \text{fat-free mass (kg)}/\text{height (m)}^2$$

Abdominal fat mass was obtained based on the manufacturer's instructions. Briefly, the android fat region was initially determined by an automatically defined region of interest box that was placed just above the iliac crest. The automatically determined height of the region of interest box was 20% of the distance from the top of the iliac crest to the base of the skull while in the supine position. Visceral fat mass was estimated within the visceral cavity and excluded the transverse abdominis based on the manufacturer's software. If necessary, fine adjustments were made to the region of interest boxes by a single researcher (WB) to more accurately discriminate between the subcutaneous layer, the transverse abdominis, and the visceral cavity. Studies have reported a moderate to strong correlation between visceral fat measured using DXA and the gold standard, computed tomography, suggesting that DXA provides an accurate estimate of abdominal fat (Kaul et al., 2012; Micklesfield et al., 2012). Visceral fat mass index (VFMI) was determined in the same manner as FMI and FFMI, as follows:

$$VFMI = \text{visceral fat mass (kg)}/\text{height (m)}^2$$

Physical Activity

Physical activity was assessed using the Actigraph GT9X (Pensacola, FL; n = 12 children with CP and n = 13 typically developing children) or the Actical (Respironics Inc., Bend, OR; n = 18 children with CP and n = 17 typically developing children) accelerometer-based physical activity monitors. The Actigraph GT9X utilizes a gyroscope, magnetometer, a triaxial MEMS

accelerometer, and measures acceleration between ± 8 g at a sampling rate of 30 to 100 Hz. The Actical activity monitors have an omnidirectional sensor that measures acceleration between 0.05 and 2 g at sampling rate of 32 Hz. The raw data mode was in 15 second epochs which are used to register activity counts (John & Freedson, 2012). To convert total activity counts from the Actical to the Actigraph monitor, a calibration equation was developed using data from 7 ambulatory children with mild CP and 9 typically developing children 4 - 11 years of age who wore both monitors on the same ankle and hip for four days (Ankle: Actigraph total activity counts = 2.9681 x Actical activity counts + 199039; r^2 = 0.961; Hip: Actigraph total activity counts = 2.7096 x Actical activity counts + 144259; r^2 = 0.913).

Participants were asked to wear two monitors on the lateral aspect of the ankle and two monitors on the hip of the more affected side in children with CP and on the non-dominant side in typically developing children. Physical activity data was recorded for four days (three weekdays and one weekend day) while the participants wore these monitors continuously for 24 hours. Participants and participant's families were asked to take the monitors off during bathing, showering, or swimming. This was confirmed by reviewing activity logs kept by the children with assistance from their parent and by visually examining the graphical output generated using software provided by the manufacturer. If participants did not wear the monitors on any of the days, they were asked to re-wear the monitors to make up for missed days. The total activity counts per day averaged from the two monitors are reported.

Blood Analysis

Fasting blood samples were collected by a trained phlebotomist. Serum was separated using standard procedures and stored at -80 °C until analysis. Total cholesterol (intra-assay CV%

= 1.33, inter-assay CV% = 4.28), high-density lipoprotein cholesterol (HDL-C) (intra-assay CV% = 6.1, inter-assay CV% = 6.57), and triglycerides (intra-assay CV% = 1.11, inter-assay CV% = 4.28) were measured on a Stanbio Sirus (Boerne, TX) analyzer, and low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's formula (Friedewald, Levy, & Fredrickson, 1972):

$$\text{LDL-C (mmol/L)} = \text{total cholesterol} - \text{HDL-C} - \text{TG}/2.2.$$

Non-high-density lipoprotein cholesterol (non-HDL-C) was calculated as follows:

$$\text{Non-HDL-C} = \text{total cholesterol} - \text{HDL-C}.$$

Glucose was measured on a Stanbio Sirus (Boerne, TX) analyzer using glucose oxidase (intra-assay CV% = 1.28, inter-assay CV% = 4.48). Insulin (intra-assay CV% = 1.49, inter-assay CV% = 3.95) was measured on a TOSOH Bioscience AIA900 (South San Francisco, CA) using immunofluorescence. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following equation:

$$[\text{fasting insulin (uU/ml)} \times \text{fasting glucose (mg/dl)}]/405$$

Total adiponectin (intra-assay CV% = 2.26, inter-assay CV% = 8.61) and high molecular weight (HMW) adiponectin (intra-assay CV% = 2.14, inter-assay CV% = 8.74) were measured in duplicate using ALPCO (Salem, NH) HMW & Total Adiponectin ELISA. Leptin was measured in duplicate using Millipore (Billerica, MA) Human Leptin RIA kits (intra-assay CV% = 4.50).

Statistical Analysis

Data were analyzed using SPSS version 24.0 (IBM Corp., Armonk, NY). All variables were checked for normality by examining skewness, kurtosis and the Shapiro–Wilk test. Group differences between children with CP and controls were determined using an independent t-test

if the data were normally distributed and a Mann–Whitney U-test if the data were non-normally distributed. Non-normally distributed variables included height, height percentile, weight, BMI, BMI percentile, total fat mass, FMI, FFMI, visceral fat mass, VFMI, HOMA-IR, triglycerides, insulin, and leptin. One sample t-tests were used to determine whether the height, weight, and BMI percentiles were different from the 50th age- and sex-based percentile in the children with CP and in the controls. Values are presented as mean ± SD unless stated otherwise. Alpha level was set at 0.05. All tests were two tailed. The magnitude of the effects were determined using Cohen's d (*d*), with 0.2, 0.5 and 0.8 representing small, moderate and large effect sizes, respectively (Cohen, 1988). Chi-squared tests were used compare prevalence of borderline-high/low and high/low total cholesterol and lipoprotein levels as outlined by the National Heart, Lungs, and Blood Association (Expert Panel on Integrated Guidelines for Cardiovascular, Risk Reduction in, Adolescents, National Heart, & Blood, 2011), as well as the prevalence of pre-diabetes as outlined by the American Diabetes Association (American Diabetes, 2014), between groups. For dyslipidemia, chi-squared tests were conducted between values categorized as acceptable and a category consisting of pooled borderline-high/low + high/low values. This was done due to the fact that some high/low categories did not contain any values.

Bivariate linear regression was used to determine the relationships between adiposity indices (i.e., visceral fat mass, VFMI, and FMI) and biochemical markers of CMD. Multiple linear regression analysis was used to determine the amount of variance in biochemical markers of CMD (non-HDL-C, glucose, HOMA-IR, and leptin) that was explained by adiposity indices (visceral fat mass, VFMI, and FMI). A focus was placed on non-HDL-C as a measure of dyslipidemia, and glucose and HOMA-IR as measures of insulin resistance, because these two conditions are central, along with visceral adiposity, to the metabolic syndrome and CMD

development (Eckel et al., 2010). Leptin has been implicated in the relationship between obesity and the metabolic syndrome (Correia & Rahmouni, 2006; Patel, Reams, Spear, Freeman, & Villarreal, 2008), and was therefore included in linear regression models involving other biochemical markers and indices of adiposity to test for interactions. All independent predictors were examined for interactions. If they were not significant contributors, they were removed from the final model.

Results

Physical Characteristics, Body Composition, and Physical Activity

Physical characteristics, body composition, and physical activity are reported in **Table 1.** Thirty children with CP and 30 control children, 5 to 11 years of age, matched for age, sex, and race (27 % female, 73 % male, 7 % Asian, 10 % black, and 83 % white) who participated in this cross-sectional study were analyzed. There were no group differences in age, sex, height, weight or BMI. Children with CP compared to controls had lower age- and sex-based height percentile ($p = 0.012$). Children with CP were significantly lower than the 50th age- and sex-based percentile for height ($p = 0.001$), but were not different for weight or BMI (both $p > 0.30$). Controls were not different from the 50th age- and sex-percentile for height, weight, or BMI (all $p > 0.70$). Children with CP had significantly higher VFMI and FMI than controls ($p < 0.05$). Children with CP also had higher visceral fat mass, as indicted by a moderate group effect ($d = 0.526$), but the difference was marginally insignificant ($p = 0.076$). Physical activity counts were 38 % lower in children with CP than controls ($p < 0.001$).

Biochemical Markers

Results from the biochemical analysis of blood, including dyslipidemia and pre-diabetes classifications and cut-off values, are reported in **Table 2**. Significantly higher total cholesterol, LDL-C, non-HDL-C, glucose, and leptin were observed in children with CP compared to controls ($p < 0.05$). Children with CP also had higher insulin and HOMA-IR, as indicated by weak-to-moderate effect sizes ($d > 0.37$), but the differences did not reach statistical significance ($p > 0.15$). Compared to typically developing children, more than 2 x as many children with CP had borderline-high or high total cholesterol (60 vs. 27 %, $p < 0.001$), 4.5 x as many children with CP had borderline-high or high LDL-C (30 vs. 7 %, $p = 0.020$), and 5.5 x as many children with CP had borderline-high or high non-HDL-C (37 vs. 7 %, $p = 0.005$). The proportion of children with CP and controls with borderline-low or low HDL-C (7 % and 10 %, respectively, $p = 0.640$) and borderline-high or high triglycerides (37 and 33 %, respectively, $p = 0.712$), was similar. Compared to controls, 8 x more children with CP presented with pre-diabetes (27 vs 3 %, $p = 0.011$).

Relationship between Adiposity Indices and Biochemical Markers of CMD

When the relationships between adiposity indices (i.e., visceral fat mass, VFMI, and FMI) and select biochemical markers (i.e., non-HDL-C, glucose, HOMA-IR and leptin) were examined, there was a significant group interaction for the relationship between visceral fat mass and HOMA-IR ($p = 0.011$). Whereas, there was a significant relationship between visceral fat mass and HOMA-IR in children with CP ($r = 0.811$, $p < 0.001$), there was no significant relationship in controls ($r = 0.255$, $p = 0.173$). For the other adiposity indices, there were no significant group interactions (all $p > 0.05$). In the combined sample, visceral fat mass, VFMI,

and FMI were all positively related to non-HDL-C ($r = 0.316$, 0.337 and 0.290, respectively, all $p < 0.05$), glucose ($r = 0.381$, 0.313 and 0.388, respectively, all $p < 0.05$), and leptin ($r = 0.714$, 0.611 and 0.915, respectively, all $p < 0.001$). Visceral fat mass index and FMI were positively related to HOMA-IR ($r = 0.583$ and 0.671, respectively, both $p < 0.001$). The positive relationships of VFMI and FMI with HOMA-IR were no longer statistically significant when leptin was included in the regression model (both $p > 0.05$).

Scatter plots demonstrating the relationships between adiposity indices (i.e., visceral fat mass an FMI) and biochemical markers (i.e., non-HDL-C, glucose, HOMA-IR and leptin) are presented in **Figure 1** and group-specific relationships are reported. Visceral fat mass was positively related to non-HDL-C in children with CP, though it was marginally insignificant ($r = 0.345$, $p = 0.062$). The relationship was weaker and not statistically significant in controls ($r = 0.055$, $p = 0.770$). Visceral fat mass was positively and significantly related to glucose in children with CP ($r = 0.498$, $p = 0.005$), but not in controls ($r = 0.126$, $p = 0.503$). Visceral fat mass was positively and significantly related to leptin in children with CP ($r = 0.784$, $p = 0.005$) and controls ($r = 0.498$, $p = 0.005$), through the strength of the relationship was greater in the children with CP. Total body fat mass index was positively related to glucose and children with CP ($r = 0.336$, $p = 0.070$) and controls ($r = 0.373$, $p = 0.042$), though the relationship was statistically significant only in controls. Total body fat mass index was also positively related to HOMA-IR and leptin in children with CP ($r = 0.677$ and 0.921, respectively, $p < 0.001$) and controls ($r = 0.601$ and 0.885, respectively, $p < 0.001$).

Discussion

The primary observation in the present study was that ambulatory children with CP demonstrated early signs of CMD. Specifically, biochemical markers reflected a higher degree of dyslipidemia, pre-diabetes, and hyperleptinemia in children with CP when compared to matched typically developing children. These unique observations are consistent with previous studies that reported a higher rate of CMD in adults with CP (Strauss et al., 1999). In addition, the results suggest that the onset of CMD in individuals with CP is during childhood, earlier than had been previously documented. Another novel finding in the present study was that the biochemical markers of dyslipidemia, pre-diabetes, and hyperleptinemia were related to the higher levels of visceral adiposity in children with CP. The findings are important because CP is associated with an increased prevalence of mortality related to CMD (Strauss et al. 1999).

This is the first study to report evidence of dyslipidemia in children or adults with CP using a control group. The typically developing control children in the present study were carefully matched to the children with CP for age, sex and race. Signs of dyslipidemia in children with CP included higher total cholesterol, LDL-C and non-HDL-C, with no difference in HDL-C. Furthermore, compared to control children, 2, 4.5 and 5.5 times more children with CP were in the borderline-high and high categories for total cholesterol, LDL-C and non-HDL-C, respectively. Perhaps the strongest indicator of dyslipidemia in children with CP was their higher levels of non-HDL-C. There is evidence that non-HDL-C, which is an estimate of all apolipoprotein B containing lipoproteins, is a more robust measure of cardiovascular disease risk (Packard & Saito, 2004) than LDL-C alone (Cui et al., 2001). The observations in children with CP are consistent with reports of dyslipidemia in young adults with CP (Ryan, Crowley, Hensey, McGahey, & Gormley, 2014; D. G. Whitney et al., 2018). In a study by Ryan et al, dyslipidemia,

as reflected by elevated LDL-C, was present in 27 % of ambulatory and nonambulatory adults with CP (Ryan, Crowley, et al., 2014). In the present study, 30 % of the children with CP and 7 % of the controls had borderline-high or high LDL-C.

This is also the first study to assess the presence of pre-diabetes in children with CP using a control group of typically developing children. Signs of pre-diabetes in children with CP were reflected by their higher levels of fasting blood glucose. Moreover, using the fasting glucose cut-off of ≥ 100 mg/dl, there were 8 times more children with CP than typically developing children who were classified as pre-diabetic. While HOMA-IR, a measure of insulin resistance, was not statistically different between groups ($p = 0.171$), it was higher on average in children with CP and the group difference approached a moderate effect size ($d = 0.404$). Previous studies have not detected glucose dysregulation in adults with CP (Ryan, Crowley, et al., 2014; D. G. Whitney et al., 2018). Some limitations of these previous studies include the lack of a control group (Ryan, Crowley, et al., 2014), or the use of data collected from medical records (D. G. Whitney et al., 2018). In the present study, the control participants were carefully matched to the children with CP. Furthermore, all participants in the present study enrolled in the study, followed the same protocol, and used all of the same preparation, collection, storage and analysis procedures.

The signs of dyslipidemia and pre-diabetes in the children with CP may be tied to the high level of visceral and total adiposity, which is supported by the observation that higher non-HDL-C and LDL-C in children with CP were accompanied by significantly higher VFMI and FMI, as well as higher visceral fat mass, though not statistically significant. Additionally, there was a modest relationship between non-HDL-C, a robust biochemical marker of dyslipidemia, and visceral fat mass and VFMI, markers of visceral adiposity, as well as FMI, a marker of total

body adiposity, in the combined sample of children with CP and control children. It has been previously reported that the relationship between visceral adiposity and dyslipidemia is stronger in those with visceral fat values above the mean than in those with values below the mean (Kelly et al., 2014). This is supported by the stronger relationship shown between non-HDL-C and visceral fat mass in children with CP, even though the relationship only reached significance in the combined sample. Together, these observations suggest a threshold of visceral adiposity, beyond which brings about a disproportionate increase in blood lipids related to CMD risk.

The present study also suggests that that visceral adiposity may be more predictive of insulin resistance and pre-diabetes in children with CP than in typically developing children. A positive relationship between measures of visceral adiposity and HOMA-IR was observed in children with CP, but not controls. This could be a function of the greater visceral adiposity observed in children with CP, as visceral adiposity that is above average displays stronger positive relationships with HOMA-IR, than those at or below the mean (Kelly et al., 2014). If visceral adiposity has a different relationship with HOMA-IR in children with CP compared to typically children, it is plausible that the underlying mechanism is an increased deposition of visceral fat in and around the liver, pancreas, and other important organs in those with CP, which is related to both dyslipidemia and insulin resistance (Gaggini et al., 2013; Samuel et al., 2004). Further research is necessary to determine the degree in which visceral adiposity accumulates within and around vital organs like the liver, pancreas, and heart in children with CP. Specifically, whether this accumulation is a function of increased visceral adiposity in general (i.e., a threshold exists in which visceral adiposity begins to selectively accumulate around vital organs), or children with CP have a greater tendency to store visceral fat around major organs due to some other mechanism, warrants investigation.

The positive relationship between serum leptin and body fatness in children with CP in the present study is consistent with previous studies on children with CP and is likely indicative of leptin resistance (Munzberg et al., 2005; Myers, Cowley, & Munzberg, 2008; Yakut et al., 2006); however, to our knowledge, the present study is the first to report this in a sample consisting only of children with mild CP. In an earlier study by Yakut et al., 72.5% of the children with CP were non-ambulatory. This study classified children with CP, but not controls, into subgroups of low and normal subcutaneous fat levels, finding that while leptin levels of the children CP groups combined were higher on average than controls, leptin levels were only elevated in the group with normal levels of subcutaneous fat. Our matched sample provides a much more direct comparison between children with CP and their typically developing peers.

In the present study, both visceral and total adiposity were strongly positively related to leptin levels. In mice, leptin administration has been shown to selectively decrease visceral fat accumulation through changes in hepatic gene expression, (Barzilai et al., 1997); therefore, increased levels of both leptin and visceral adiposity indirectly suggest a suppression of leptin's effects in those with CP. This may also provide a mechanism for the increased visceral adiposity observed in children and adults with CP in previous studies (Daniel G. Whitney et al., 2018; Peterson et al., 2015). Additionally, the relationships between measures of visceral and total body adiposity with insulin resistance were no longer significant when leptin was statistically controlled, suggesting that leptin moderates this relationship. However, considering the small size of the sample in the current study, such an inference is stated with caution. Nonetheless, our finding that leptin is significantly related to insulin resistance in both groups supports this notion. Leptin's influence on insulin resistance is logical considering that leptin exerts counter-regulatory actions on both insulin production and its receptors that aid in maintaining insulin

sensitivity in the liver (Barzilai et al., 1999) and skeletal muscle (Ceddia, William, & Curi, 1999); therefore, the blunting of leptin's effects (i.e. leptin resistance) can contribute to insulin resistance (Levi et al., 2011). It also follows that leptin resistance is a plausible contributor to the increased intramuscular adiposity previously reported in children and adults with CP (D'Souza et al., 2020; Johnson et al., 2009; Noble et al., 2014; D. G. Whitney et al., 2017), as leptin has been shown to increase oxidation and decrease storage of fatty acids in lean, but not obese, human skeletal muscle (Steinberg, Parolin, Heigenhauser, & Dyck, 2002).

An important strength of this study in relation to similar previous studies is not only the presence of a control group, but also that the children with CP were matched to typically developing children for age, sex, and race. Moreover, the typically developing children were not different from the 50th age- and sex-based percentiles for height, weight, and BMI. This provides evidence that the controls were reasonable representatives of the general population of children. The observation of an elevated CMD risk in a sample of non-obese, ambulatory children with a mild form of CP is especially novel as most research on CMD risk in children involves those with obesity.

The limitations of the study must also be discussed. While, to our knowledge, this is the first study to assess CMD risk using biochemical markers of dyslipidemia and insulin resistance and their relationship with visceral adiposity in children with CP compared to typically developing children, only relationships can be established due to the cross-sectional design of the study. Further research is needed to establish causality, and even the direction of these relationships, as many of them are complex and likely bi-directional. The study also employed DXA to assess visceral fat, which does not provide the same level of accuracy as other methods, such as magnetic resonance imaging and computed tomography. Nonetheless, studies have

demonstrated that DXA provides valid estimates of visceral fat mass (Kaul et al., 2012; Micklesfield et al., 2012). Moreover, statistically significant relationships between DXA measures of visceral fat and biochemical markers of CMD risk were observed despite these methodological limitations.

In conclusion, ambulatory children with CP display early signs of dyslipidemia, pre-diabetes, and leptin resistance, which are related to visceral adiposity. To better assess CMD risk in children with CP, the thresholds for traditional markers, such as BMI, may need adjustment. An assessment protocol that includes biochemical markers and more extensive measures of body composition may also be warranted. Moreover, studies that identify strategies to suppress the early development of CMD in children with CP are needed.

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Tables & Figures

Table 1. Physical characteristics, body composition, and physical activity of children with cerebral palsy (CP) and typically developing children (Con)

	CP (n = 30)	Con (n = 30)	*d*	*p*
Age (y)	8.7 ± 2.3	8.7 ± 2.0	> 0.001	0.919
Sex (M/F)	22/8	22/8		
Race (White/Black/Asian)	25/3/2	25/3/2		
Height (m)	1.27 ± 0.15	1.31 ± 0.13	0.285	0.239
Height (%)	29.8 ± 31.0*	50.5 ± 30.4	0.674	0.009
Weight (kg)	29.3 ± 9.7	29.4 ± 9.2	0.011	0.963
Weight (%)	44.0 ± 34.6	50.0 ± 31.7	0.181	0.491
BMI (kg/m^2)	17.8 ± 3.8	16.7 ± 2.8	0.330	0.201
BMI (%)	56.8 ± 36.0	48.1 ± 32.9	0.252	0.335
GMFCS (I/II/III)	19/9/2			
Visceral fat mass (kg)	0.22 ± 0.14	0.16 ± 0.08	0.526	0.076
VFMI (kg/m^2)	0.14 ± 0.08	0.09 ± 0.05	0.750	0.018
Total fat mass (kg)	8.98 ± 5.47	7.13 ± 4.00	0.306	0.249
FMI (kg/m^2)	5.32 ± 2.72	4.03 ± 1.82	0.557	0.049
Fat free mass (kg)	19.5 ± 6.1	21.0 ± 5.9	0.250	0.442
FFMI (kg/m^2)	11.9 ± 1.9	11.9 ± 1.4	0.010	0.355
Physical activity (counts)	1280151 ± 649378	2062677 ± 589832	1.261	> 0.001

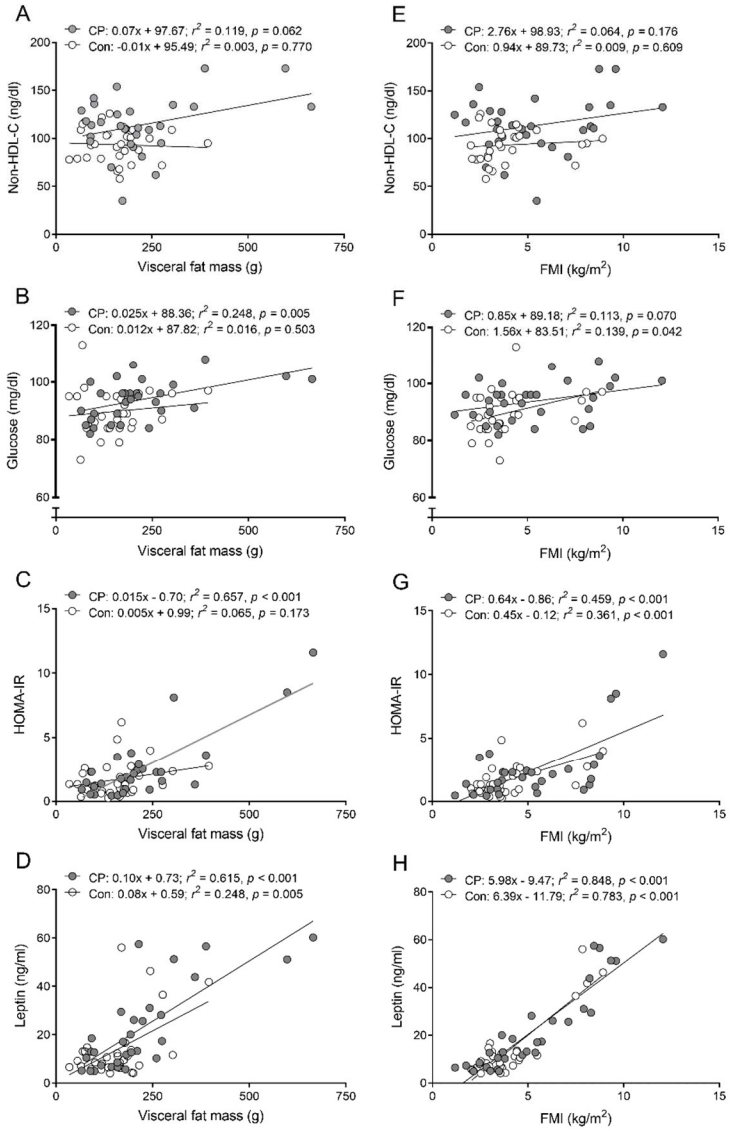
Values are mean ± SD; % for height, body mass, and body mass index (BMI) reflect the percentile relative to age- and sex-based norms; GMFCS = gross motor function classification system. VFMI = visceral fat mass index; FMI = total body (minus head) fat mass index); FFMI = total body (minus head) fat-free mass index; *Different from age- and sex-based 50^{th} percentile.

Table 2. Biochemical analysis of children with cerebral palsy (CP) and typically developing children (Con)

	CP (n = 30)	Con (n = 30)	*d*	*p*
Total cholesterol (mg/dl)	174 ± 32	154 ± 22	0.728	0.006
Acceptable (< 170)	12 (40%)	22 (73.3%)		
Borderline-high (170-199)	14 (46.7%)	6 (20%)		
High (≥ 200)	4 (13.3%)	2 (6.7%)		
HDL-C (mg/dl)	60.8 ± 10.2	61.0 ± 11.1	0.019	0.933
Acceptable (> 45)	28 (93.3%)	27 (90%)		
Borderline-low (40-45)	2 (6.7%)	2 (6.7%)		
Low (< 40)	0 (0%)	1 (3.3%)		
LDL-C (mg/dl)	96.1 ± 25.8	78.2 ± 18.4	0.799	0.003
Acceptable (< 110)	21 (70%)	28 (93.3%)		
Borderline-high (110-129)	8 (26.7%)	2 (6.7%)		
High (≥ 130)	1 (3.3%)	0 (0%)		
Non-HDL-C (mg/dl)	113.6 ± 29.6	93.5 ± 17.6	0.825	0.002
Acceptable (< 120)	19 (63.3%)	28 (93.3%)		
Borderline-high (120-144)	8 (26.7%)	2 (6.7%)		
High (≥ 145)	3 (10%)	0 (0%)		
Triglycerides	87.1 ± 85.2	76.4 ± 42.5	0.159	0.684
Acceptable (< 75[a], < 90[b])	19 (63.3%)	20 (66.7%)		
Borderline-high (75-99[a], 90-129[b])	5 (16.7%)	4 (13.3%)		
High (≥ 100[a], ≥ 130[b])	6 (20%)	6 (20%)		
Glucose (mg/dl)	93.7 ± 6.9	89.8 ± 7.6	0.537	0.042
Acceptable (< 100)	22 (73.3%)	29 (96.7%)		
Pre-diabetic (100-125)	8 (26.7%)	1 (3.3%)		
Insulin (uU/ml)	10.5 ± 10.1	7.4 ± 5.7	0.378	0.181
HOMA-IR	2.53 ± 2.56	1.70 ± 1.37	0.404	0.171
Total adiponectin (ng/ml)	8.96 ± 2.45	8.71 ± 2.56	0.010	0.695
HMW adiponectin (ng/ml)	4.06 ± 1.60	3.78 ± 1.61	0.174	0.237
Leptin (ng/ml)	22.3 ± 17.7	13.9 ± 13.2	0.538	0.027

Values are mean ± SD; Acceptable, borderline-high/low and high/low lipid and lipoprotein levels established by the Expert Panel on Integrated Cardiovascular Health and Risk Reduction in Children and Adolescents (Expert Panel on Integrated Guidelines for Cardiovascular et al., 2011); 0-9 y[a], 10-19 y[b]. Acceptable and pre-diabetic glucose levels are based on the American Diabetes Association "Standards of Medical Care in Diabetes (American Diabetes, 2014)

Figure 1. Relationships between visceral fat mass (A-D), fat-free mass (FMI; E-H) and biochemical markers in children with cerebral palsy (CP) and typically developing children (Con)



Separate regression lines are plotted for CP and Con for all relationships. Combined regression coeffecients for relationships in which a group interaction was not detected are reported in the results section. Non-HDL-C = Non high-density lipoprotein cholesterol, FMI = Fat mass index, HOMA IR = Homeostatic Model Assessment of Insulin Resistance

CHAPTER 4

CONCLUSIONS AND SUMMARY

The primary observation in this study was that children with CP had an increased prevalence of dyslipidemia, as indicated by higher total cholesterol, LDL-C, and non-HDL-C (with no difference in HDL-C) and a higher prevalence of pre-diabetes, as indicated by higher fasting blood glucose, compared to typically developing children. Visceral adiposity was positively related to these CMD risk factors. Children with CP also had hyperleptinemia, likely indicative of leptin resistance. The study results suggest that the elevated risk factors of CMD previously observed in young and older adults (Heyn et al., 2019; D. G. Whitney et al., 2018) are present during childhood. Clinically, the results of this study should provide context for care providers to better screen for CMD risk in children with CP. Care providers should be informed of the increased prevalence of dyslipidemia, pre-diabetes, and hyperleptinemia in this population, and take steps to lessen the burden of CMD. Lastly, this study opens the door for further research into nutritional strategies, exercise strategies, and other lifestyle interventions that can best mitigate this higher CMD risk in children with CP than in typically developing children.

CHAPTER 5

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