Secondary muscle pathology and metabolic dysregulation in adults with cerebral palsy

Mark D. Peterson,1 Paul M. Gordon,1 Edward A. Hurvitz,1 and Charles F. Burant2,3
1Department of Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, Michigan; 2Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan; and 3Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan

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Secondary muscle pathology and metabolic dysregulation in adults with cerebral palsy. Am J Physiol Endocrinol Metab 303: E1085–E1093, 2012. First published August 21, 2012; doi:10.1152/ajpendo.00338.2012. —Cerebral palsy (CP) is caused by an insult to or malformation of the developing brain which affects motor control centers and causes alterations in growth, development, and overall health throughout the life span. In addition to the disruption in development caused by the primary neurological insult, CP is associated with exaggerated sedentary behaviors and a hallmark accelerated progression of muscle pathology compared with typically developing children and adults. Factors such as excess adipose tissue deposition and altered partitioning, insulin resistance, and chronic inflammation may increase the severity of muscle pathology throughout adulthood and lead to cardiometabolic disease risk and/or early mortality. We describe a model of exaggerated health risk represented in adults with CP and discuss the mechanisms and secondary consequences associated with chronic sedentary behavior, obesity, aging, and muscle spasticity. Moreover, we highlight novel evidence that implicates aberrant inflammation in CP as a potential mechanism linking both metabolic and cognitive dysregulation in a cyclical pattern.}

CEREBRAL PALSY (CP) IS OFTEN DESCRIBED as the most common physical disability of childhood, affecting between 2 and 3.6 per thousand live births (49, 73, 125). Over the past few years, there has been greater awareness of the unique problems facing children with CP as they transition to adulthood. CP is caused by a malformation or insult to the developing brain which affects motor control centers and causes alterations in growth, development, and overall health and function throughout the life span. Damage can occur during pregnancy, childbirth, or after birth up to about age three. Once established, the brain insult or structural problems do not appear to progress with time, but individuals with CP are subject to a number of secondary conditions that may interfere with important aspects of quality of life, such as independence, participation, and employment (11, 64). These secondary complications can impede the monitoring of health risks as well as the diagnosis and treatment of medical complications.

Unfortunately, at present there is a divergence between the basic research intended to uncover novel etiologic factors and treatments of CP and that which occurs at the translational level to understand secondary mechanisms or complications unique to this population. Clinical studies tend to focus on common symptoms, such as spasticity, gait disorders, pain, and fatigue, as well as the efficacy and outcomes of respective medical interventions. Conversely, most mechanistic research in this population is limited to prenatal and perinatal abnormalities and developmental neurology. Despite the significant progression of disability that is known to occur, there has been very little focus on understanding the etiology of comorbid conditions in adults with CP, independent of those exerted by the neurological insult. Considering that adults with mild to moderate CP may have relatively normal life expectancy, it is critical to unravel the discrete mechanisms of secondary disability and comorbidity.

Muscle Pathology and Cardiometabolic Risk in CP

Individuals with CP have permanent neurological impairment that compromises motor function, mobility, and balance. In conjunction with reduced muscle mass (52), these factors may predispose young or middle-aged adults with CP to sustain functional declines similar to those seen in older adults without CP (99). Secondary to the underlying neurological insult, individuals with CP have a documented inability to maximally recruit target muscle during voluntary activity and an overrecruitment and coactivation of antagonist muscle (88, 99, 104). These motor control impairments dramatically reduce gait and movement efficiency and increase energy expenditure and fatigue during tasks (51, 61, 62, 79, 119). Premature declines in function among adults with CP (11, 19, 40, 70, 116) may occur as a result of early and accelerated sarcopenia and weakness beyond that which is expected for typical aging adults (99).

While the specific mechanisms of secondary muscle pathology and comorbidities (116) are not well defined, ample evidence exists to confirm that individuals with CP have lower fitness (23, 68), less muscle mass (52), diminished bone density (26, 97), neuromuscular inefficiency (88, 99, 104), and reduced functional reserve throughout the span of adulthood. Along with the hallmark motor impairments, pronounced sedentary behavior and fitness deficits that occur in CP (60) have prompted a comparison model of disability related to spinal cord injury (6), a population with significant muscle atrophy, increased adiposity, insulin resistance (IR), hyperlipidemia, and elevated prevalence of type 2 diabetes (7). However, individuals with CP have the added risk of secondary muscle pathology and metabolic dysregulation from birth. This leads to an ongoing circular series of events that result in debilitating losses of muscle function and an exaggerated risk for cardiometabolic disease (Fig. 1). Indeed, mortality records have demonstrated a two- to threefold greater death rate from coronary heart disease among adults with CP compared with
the general population (106). Presently, there are no national surveillance programs that monitor CP patients longitudinally. However, recent cross-sectional data demonstrate that overweight/obese adolescents with CP have a higher prevalence of dyslipidemia, hypertension, and fatigue than age- and weight-matched individuals without CP (83).

Most research pertaining to obesity among individuals with CP has been conducted to characterize prevalence (36, 85) or to compare and cross-validate anthropometric and body composition strategies (33, 120). Several studies have identified a general, increased prevalence of obesity among children with CP (36, 85), but this has yet to be documented in adults. While most studies have focused on measurement strategies for relative adiposity or the validation of methods to predict body fat stores among patients with CP (33), findings also reveal significantly diminished body “protein” (4) and specific muscle tension (i.e., torque/cross-sectional area) (22), and thus highlight the implications of skeletal muscle pathology. Since individuals with CP have diminished lean body mass, even normal BMIs in this population may conceal excessive body fat and/or elevated cardiometabolic risk, i.e., “normal-weight obesity” (86).

Myosteatosis, Mitochondrial Dysfunction and Insulin Resistance

Intermyocellular accumulation of adipose tissue (IMAT; i.e., extracellular) and intramyocellular lipid (IMCL) and reductions in mitochondrial size, density, and function (75, 78, 94) have been implicated in the etiology of IR, the metabolic syndrome, and diabetes. Ample debate pertaining to the nature of these associations has raised questions about directional link, i.e., whether mitochondrial dysfunction and/or myosteatosis leads to IR or vice versa (43, 71, 75), as well as the contributing role of “intrinsic function” or content of mitochondria in the pathogenesis of cardiometabolic dysregulation (77, 107). Moreover, age-related decreases in mitochondrial function are also thought to give rise to accumulation of IMCL and may thus represent a pathophysiological mechanism for the hallmark IR among many older adults (90). These observations have prompted comparison between aged muscle and obese muscle and hint at a common mechanistic link related to lipotoxicity within/around the myocyte.

Previous studies that have suggested elevated IMAT or IMCL content in obesity, diabetes, and aging have used magnetic resonance imaging and spectroscopy, which do not provide detail regarding subcellular localization, ultrastructure, or proximity to mitochondria within skeletal muscle (18, 74). Recent quantitative electron microscopy by Chomentowski et al. (15) suggests that, among obese, insulin-resistant, and diabetic individuals, intermyofibrillar mitochondrial content is decreased in situ compared with lean, insulin-sensitive subjects, whereas subsarcolemmal subpopulations and overall mitochondrial size were found to be similar between groups. Across the continuum of insulin sensitivity between groups, intermyofibrillar mitochondrial content was positively associated with increased insulin sensitivity, fasted whole body respiratory quotient, and metabolic flexibility, the latter defined
as the difference between steady-state and fasting respiratory quotient (i.e., by indirect calorimetry during euglycemic clamps). In contrast, a recent study examining the effect of age on mitochondrial and lipid ultrastructure, Crane et al. (17) found that older adults have a significantly reduced mitochondrial density in the subsarcolemmal region compared with activity-matched young adults. This decrease was coincident with reduced mitochondrial enzyme activity (i.e., citrate synthase and cytochrome c oxidase) and suggests that aging may also result in fewer lipid droplets touching mitochondria in the subsarcolemmal region in men and throughout the entire muscle fiber in women.

The specific defects in mitochondrial content or function with obesity and IR may well be distinct from that which occurs during normal aging. However, considering the prevalence of muscle atrophy and sedentary behavior among individuals with CP, as well as the hallmark decline in “functional reserve” and fatigue during activity, it is certainly possible that several mechanistic defects may be occurring in these patients. Secondary factors such as increased skeletal muscle fat deposition or decreased mitochondrial function may serve to exaggerate the severity of functional impairment throughout adulthood, which could result in decreased muscle insulin sensitivity and exacerbate cardiometabolic risk. Identifying the degrees of IMAT, IMCL, and mitochondrial structure and function alteration in individuals with CP at different ages and its relation to IR will help define the progressive risk for this population and guide the approach and aggressiveness for treatment.

**Lipid Mediators, Oxidative Stress, Inflammation, and Extracellular Matrix: a Plausible Link in CP**

Recent studies have provided evidence that IR may not arise from fatty acid availability or skeletal muscle fat accumulation per se, but rather from the fate of fatty acids delivered to the muscle. Obesity is associated with increased supply of fatty acids to the muscle; however, it is unlikely that mitochondrial function could be impaired to such an extent that would prevent muscle from oxidizing fatty acids for typical day-to-day energy demands (69). This notion is buttressed by studies that have demonstrated an increased oxidation rate among obese and insulin-resistant compared with lean individuals (14, 38), which likely reflects the increased fatty acid flux to the muscle. The increased delivery may exceed the oxidative needs of the myocyte to generate ATP, especially in resting muscle. Excess fatty acids can accumulate as intracellular triglyceride and, if this storage is exceeded, an increased formation of other lipid intermediates (i.e., fatty acyl-CoA, diacylglycerol, ceramides and lipid peroxidation products) will occur (67), which triggers cellular responses leading to IR. Thus, the accumulation of IMCL may simply serve as a surrogate marker for fatty acid flux that exceeds oxidation but would parallel the formation of signaling lipids (1, 67). Moreover, regardless of oxidative capacity, skeletal muscle lipid will accumulate whenever the supply of fatty acids exceeds cellular energy demand (69).

Some studies have suggested that mitochondrial fatty acid availability that is in excess of the capacity for complete oxidation could lead to mitochondrial stress and IR (50). Indeed, elevations in the redox state coincident with an excess of metabolic fuel, in relation to demand, is known to result in leak of unstable single electrons that react with O2 to form the free radical superoxide (O2·−), and subsequent H2O2 emission (for review see Ref. 24). Although this mitochondrial “stress” is a hallmark of diabetes (2), few studies have yielded direct evidence regarding the specific species of reactive oxygen species (ROS) that mediates the development of IR. Regardless, during conditions of excess fuel delivery, mitochondria are likely the principal source of H2O2 emission to establish the intracellular redox environment (i.e., reducing vs. oxidizing). In the event that supply of reducing equivalents exceeds demand, the elevated reduction potential within the environment increases the formation of O2·−, H2O2 emission, and disruption of insulin signaling. The exact role of increased mitochondrial H2O2 emission to disrupt insulin signaling is yet to be completely elucidated; however, emerging evidence suggests that alterations in the redox environment through disruption of serine/threonine (Ser/Thr) phosphorylation of residues with the insulin receptor substrate (IRS) proteins (12) may have widespread pathophysiological implications.

There is also a growing body of evidence suggesting that inflammation may provide an important mechanistic link between obesity and IR (59, 72, 98). Mediators of inflammation have been shown to contribute to macrophage infiltration and IR in animal models (44, 121), thus implying that inflammation may precede and/or trigger the development of IR. Classically activated macrophages [i.e., proinflammatory adipose tissue macrophages (ATMs)] are widely known to secrete various cytokines such as resistin, IL-6, IL-1β, and TNFα and thus act through paracrine mechanisms to inhibit insulin action in the cell (for review see Ref. 29). While ATM-induced cytokine secretion indeed has a local influence on insulin sensitivity, there is also a well-known endocrine effect of circulating cytokines (e.g., increased activation of kinases IKKβ, JNK1, and PKCθ) to phosphorylate IRS-1 (93) and to increase ceramide biosynthesis and inhibition of Akt/PKB activation (35), both of which ultimately lead to impairment in downstream insulin signaling (29). Much attention has been devoted to the specific mechanisms linking inflammation and IR, and ample evidence now exists to support the independent role of both saturated fatty acid-induced changes in Toll-like receptor signaling [e.g., TLR4 as a receptor for lipopolysaccharide (LPS) (91)] to induce expression of proinflammatory genes (80), as well as stress kinase-induced ROS production and eventual NLRP3 inflammasome stimulation (124), a pathway that may be specific to saturated fatty acids (Fig. 2).

Indeed, chronic inflammation in CP has been considered a possible tertiary mechanism of prolonged neurological injury processes (i.e., from aberrant gliosis and impaired/delayed white matter regeneration) (25). However, and despite the extremely well-characterized neuroinflammatory response associated with periventricular leukomalacia (PVL) (81), the leading cause of spastic CP in preterm infants (48), no research has been conducted to examine the potential circulus cause and consequence of events with obesity-induced inflammation in CP. In fact, one of the only studies conducted to examine the peripheral inflammatory response revealed that seven-year-old children with PVL-induced CP had increased mRNA expression of TLR4, TNFα, JNK, TAK1, and IKKγ after LPS stimulation compared with typically developing matched controls (58). There is ample reason to believe that this altered peripheral inflammatory response in CP is at least partially...
influenced by cardiometabolic dysregulation or significantly exaggerated by it. Pediatric obesity stimulates innate immunological activation and a chronic metainflammatory response (32, 59) as early as three years of age (101). Moreover, several lines of research now indicate a role for high-fat diet to induce neuroinflammation (i.e., hypothalamic) (65, 111) and a cyclical alteration in the regulatory mechanisms associated with sympathetic drive, satiety, and nutrient storage/energy homeostasis (110) (Fig. 2). Young children are particularly vulnerable to aberrant neuroinflammation, and recent findings demonstrate that, in both humans and rodent models, early high-fat feeding and obesity may be associated with latent neuronal injury (111). New evidence out of the Häring laboratory (92) has also revealed that high-fat feeding is linked to impaired insulin action in the brain, which results in pathophysiological consequences for neuronal activity and locomotion and moreover appears to be mediated through a TLR2/4, IL-6, and osteopontin (OPN) pathway. On the other hand, there is also a well-characterized response to acute neuronal injury to stimulate adipose proinflammatory cytokine expression through the sympathetic system, leading to transient peripheral insulin resistance and hyperglycemia (123). This raises the distinct prospect that aberrant chronic inflammation, irrespective of etiology, may have a profound effect on both cardiometabolic and cognitive dysregulation in a highly vulnerable population such as children with CP. Although at present we cannot separate the effects of disrupted development due to the primary insult vs. that which may occur as a tertiary consequence of chronic inflammation, these active processes may serve as potential targets for pharmacological [e.g., sirtuins (8) and G protein-coupled receptors (109)] or behavioral treatments (e.g., physical activity) to improve long-term neurological and cardiometabolic health among individuals with CP. Future research is needed to examine specific proinflammatory pathways that are common to multiple organs, as well viable target anti-inflammatory stimuli/mechanisms/pathways. Considering the emerging findings that demonstrate the potent anti-inflammatory effects of physical activity through activation of IL-10 [i.e., a core inhibitor of IKKβ/NF-κB signaling pathways (87)], a central preventive strategy from a clinical context is to encourage lifestyles characterized by habitual physical activity and fragmented sedentary behavior.

In addition to the hallmark dysregulation of lipid flux, inflammation, and oxidative stress in obesity, lipid oversupply leads to profound increases in the mRNA levels of several extracellular matrix (ECM) genes, such as collagens, fibronectin, and proteoglycans, even in otherwise healthy individuals (9, 82). Seminal studies have now confirmed the inflammatory response associated with IR has extensive effects on ECM remodeling with the insulin-resistant skeletal muscle phenotype characterized by increased collagen content (9, 82). Proteomics analysis demonstrates there are simultaneous decreases in mitochondrial proteins and cytoskeletal/structural genes and proteins (α-actinin 2 and desmin) as well as dramatic

Fig. 2. Molecular pathways at the interface between obesity and aging in CP. This schema shows that upregulation of Toll-like receptor (TLR)/nuclear factor (NF)-κB and TLR/c-Jun NH2-terminal kinase (JNK) pathways may be involved in saturated fatty acid (SFA)- and/or lipopolysaccharide (LPS)-mediated cytokine [e.g., tumor necrosis factor (TNF)-α] expression among aging, obese adults with CP. Also depicted are the confluence and consequences of sarcopenia and obesity in CP to induce a local lipotoxic effect in muscle characterized by increased mitochondrial stress, increased (IMCL), and lipid intermediates [e.g., diacylglycerols (DAGs), triacylglycerols (TGs), acyl-CoAs], fibrosis, macrophage polarization (i.e., M1), and ultimately frailty and insulin resistance.
increases in ECM proteins (37) in insulin-resistant muscle from obese individuals. Based on these data, a novel hypothesis raises the possibility that inflammation-related changes in ECM might precipitate alterations in mitochondrial function (16). In this regard, an inflammatory response would lead to changes in ECM and in turn an alteration in mechanosignal transduction (i.e., mediated through desmin filaments or actin cytoskeleton) and gene expression for myogenesis and mitochondrial function (16). This raises the distinct possibility that inflammation-mediated changes in ECM could directly affect mitochondrial function and thus lead to altered fat oxidation, the accumulation of lipid intermediates such as fatty acyl-CoAs and ceramides, and changes to insulin signaling and/or IR (16).

The formation of ECM has also been considered a mechanistic mishap in the aging skeletal muscle regeneration process, through which adipocyte and scar tissue “infiltration” occurs instead of myogenesis (28, 113). Age-related myosteatosis (66, 108) and fibrosis (30) of muscle is now well documented in the literature as elements of sarcopenia and frailty (112), as well as in conjunction with certain disease processes (27, 54) and during extended periods of sedentary behavior (63). This attenuation of skeletal muscle has been documented longitudinally within the older adult population and is known to significantly impair gross muscle function (20). Great lengths have been taken to identify the origin of this degenerative cycle, and some evidence exists to suggest that fibrocytes and adipocytes may develop from myogenic satellite cells during an altered lineage choice and due to changes in the microenvironment associated with aging, injury, and/or disease (13, 55, 96). However, recent findings have identified distinct populations of primary fibrocyte/adipocyte progenitor cells (FAPs) (i.e., resident-mesenchymal progenitors expressing PDGRFα+) that reside in muscle and may, under inhospitable physiological conditions (e.g., oxidative stress and chronic inflammation), serve to simultaneously impair myogenesis and form fat and scar tissue within the muscle (41, 117, 118). Moreover, and in contrast to the original hypothesis, evidence now discounts the capacity of quiescent satellite cells to adopt nonmyogenic fates (105). Nevertheless, these studies do show that cross-talk between myogenic cells and those of the FAP lineage occurs in the maintenance and regeneration of skeletal muscle and, as occurs coincident with aging and disease, in the degeneration of muscle and formation of fibrosis (84).

Perhaps the most extreme example of fibrosis occurs with Duchenne muscular dystrophy (DMD), in which mutations in dystrophin render muscle susceptible to a continuous cycle of regeneration/regeneration and chronic inflammatory invasion characterized by a heterogeneous infiltration of immune cells that lead to muscle degeneration (115). During chronic damage or stress associated with repeated bouts of muscle regeneration, the persistent presence of the macrophage cell types results in increased expression of proinflammatory chemokines and cytokines such as TNFα and IL-6 (114). The cytokines can precipitate altered satellite-cell function and aberrant regeneration and progressive development of fibrosis and fat accumulation. During advanced stages of DMD, lipid-filled adipocytes and scar tissue replace the majority of dystrophic muscle tissue. Although certainly an extreme example, the mdx mouse has been widely used to study dystrophic muscle (122) and potentially serves as a viable model for understanding muscle degeneration with general age-related pathology, morbid obesity, IR, and chronic sedentary behavior. Considering the heterogeneity in the macrophage population (i.e., pro- vs. anti-inflammatory) during the muscle regeneration process (3), an imbalance of macrophage infiltration phenotypes could lead to muscle pathology. Indeed, a current line of evidence has revealed increased expression of alternatively activated macrophages (M2a) in dystrophic muscle (21), and indicates a role for macrophage polarization in repair vs. adipo/fibrotic tissue conversion (122). In conjunction with the increased expression of M2a, there seems to be a concomitant and relative decrease of CD56+ satellite cells (21). Together, these findings suggest a critical role for the satellite cell microenvironment niche in modulating adipo/fibrotic or myogenic progeny (95).

The Spastic CP Muscle: a Novel Phenotype?

In borrowing findings from obese, insulin-resistant muscle and those from aging and dystrophic muscle, it is tempting to speculate a confluence or partial overlap of phenotypic attributes represented in spastic muscle of CP (Fig. 1). Although by definition CP is a nonprogressive condition (89), muscle pathology is well known to progress with age (46). Previous immunohistochemical and biochemical assay studies have suggested aberrant adaptation of ECM (10, 57) and altered myogenic pathways (22) in CP muscle. Transcriptional analyses have implicated specific gene expressions that could explain the unique muscle phenotype in CP. Spastic muscle demonstrates a prolific increase in ECM that is coincident with altered transcripts involved in myogenesis (103). Interestingly, fiber type in spastic CP muscle is found to be predominately type II compared with normal muscle, and moreover, ontology analysis has revealed losses of metabolic and mitochondrial related transcripts as well as decreases in oxidative metabolic gene transcription (103). In building off these findings, the Lieber laboratory has recently demonstrated that contracture within spastic muscle is characterized by increased passive stiffness, increased collagen content, and increased sarcomere length compared with healthy muscle from control children (102). These findings suggest that increased passive tension in contracture is actually due to changes in ECM stiffness rather than intracellular abnormalities per se. Potential mechanisms underlying these secondary maladaptive responses may include altered expression of myostatin, which is known to be increased in CP (103), and thus could negatively regulate muscle regeneration and simultaneously elicit proliferation of fibroblasts and ECM proteins (56). It is also plausible that the chronic sarcomere strain in contracture may actually induce a perturbation of the muscle regeneration process and lead to the hallmark chronic injury response, which is characterized by increased collagen and fibrosis (5). Moreover, although the immune response has not been characterized in chronically spastic muscle among patients with CP, it would be of interest to determine the macrophage polarization profile and its potential to induce fibrosis similar to that observed in dystrophic muscle (21). However, considering the significant muscle atrophy and weakness in CP, as well as the increased risk for sedentary behavior, it is very likely that secondary pathology has numerous simultaneous mechanistic origins.
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Future Directions

In light of multiple inputs that could result in exacerbation of the primary defect in patients with CP, it will be important to consider how the vicious and synergistic cycle of oxidative stress, chronic inflammation, adipose/fibrotic formation, and diminished mitochondrial biogenesis could be modulated to ameliorate IR and subsequent metabolic abnormalities that pose risks for cardiovascular disease. Recognizing the prospect for multiple, potentially synergistic insults to the muscle, it is vital not only to identify the independent mechanistic constituents of this phenotype but also to search for evidence of overlap with other, modifiable phenotypes, such obesity and unhealthy diets, that may have a significant impact in this patient population. Determining the cellular and molecular mechanisms of secondary pathology and comorbidity will aid in the development of new therapeutic strategies for maintaining muscle mass and function, enabling global cardiometabolic health, and improving quality of life for adults with CP.

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