Adipose Tissue Infiltration of Skeletal Muscle in Children with Cerebral Palsy

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Objective To determine whether children with quadriplegic cerebral palsy (QCP) have a greater adipose tissue (AT) infiltration of skeletal muscle than typically developing children (12/group and 5-14 years).

Study design Cross-sectional area (CSA) of AT and muscle in the midthigh were assessed with magnetic resonance imaging. Physical activity was assessed with an activity monitor.

Results Children with QCP had 2.3-fold higher intermuscular AT CSA and 51% lower muscle CSA in the midthigh than control subjects. Midthigh intermuscular, subfascial, and subcutaneous AT CSA adjusted for midthigh muscle CSA were higher in children with QCP (all $P < .05$). Moreover, the proportion of intermuscular AT CSA and subfascial AT CSA relative to subcutaneous AT CSA in the midthigh were 2.5-fold and 1.8-fold higher in children with QCP than control subjects (all $P < .05$). Children with QCP also had 70% fewer physical activity counts, which was inversely related to intermuscular AT CSA ($r = -0.76$) and subfascial AT CSA ($r = -0.63$) adjusted for muscle CSA in the midthigh of children with QCP (both $P < 0.05$), but not in control subjects.

Conclusion Children with QCP have a greater AT infiltration of skeletal muscle than typically developing children, which is related to their low level of physical activity. (*J Pediatr* 2009;154:715-20)

Obesity is associated with an increased risk for chronic disease.¹ Although obesity is characterized by excess adipose tissue (AT), the chronic diseases associated with obesity are often more strongly related to the degree of AT infiltration within and around skeletal muscle than total AT.^{2,3} Children with quadriplegic cerebral palsy (QCP) have decreased mobility, fat-free mass, and energy expenditure,^{4,5} all of which have been tied to excessive AT infiltration of skeletal muscle.^{3,6-8} Because cerebral palsy results from an injury to the brain before, during, or shortly after birth, the motor skills needed to adequately engage in physical activity are severely underdeveloped in children with QCP, and extreme sedentary living typically results.⁹ Consequently, if individuals with QCP have an increased AT infiltration of skeletal muscle, it may emerge during childhood. However, the presence of excessive AT infiltration remains uncertain, because in addition to their limited physical activity, fat-free mass, and energy expenditure, many children with QCP also show signs of undernutrition because of poor oral motor skills.¹⁰

After considering the paradox of undernutrition on one hand versus the decreased mobility, fat-free mass, and energy expenditure^{4,5} and the high risk of chronic disease associated with severe cerebral palsy¹¹ on the other hand, it appears that an assessment of AT infiltration of skeletal muscle in individuals with QCP is needed. The primary purpose of this study was to determine whether children with QCP who are unable to ambulate independently have a greater AT infiltration of skeletal muscle in the midthigh than typically developing children of the same age, pubertal development, and sex. A secondary purpose was to determine whether the degree of AT infiltration of skeletal muscle was associated with physical activity assessed by using an accelerometer-based activity monitor.

METHODS

Fourteen Caucasian children with QCP who were unable to ambulate independently and 12 typically developing Caucasian children matched to the children with QCP for age, pubertal development, and sex, between the 10th and 90th percentiles for height,

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AT	Adipose tissue	GMFC	Gross Motor Functional Classification
BMI	Body mass index	MRI	Magnetic resonance imaging
CSA	Cross-sectional area	QCP	Quadriplegic cerebral palsy

body mass, and body mass index (BMI) and not engaged in organized physical activity >3 hours/week participated in the study. The institutional review boards at the University of Delaware and the AI DuPont Hospital for Children approved the study. Subjects' parents provided written consent and, when able, subjects provided written assent.

In children with QCP, forearm length was used to estimate height.¹² In control subjects, height was measured to the nearest 0.1 cm by using a stadiometer. In all children, body mass was measured to the nearest 0.1 kg by using a digital wheelchair scale (Detecto 6550, Cardinal Scale, Webb City, Missouri). Age-based percentiles for height and body mass were determined by using published data from the Center for Disease Control.¹³

Pubertal development was assessed by a physician assistant using the Tanner staging technique.¹⁴ The technique is based on a 5-point scale, with 1 indicating no development and 5 indicating full development. Pubic hair and breast development were assessed in girls. Pubic hair and penis/testicular development were assessed in boys.

The degree of gross motor functional ability was assessed with the Gross Motor Functional Classification (GMFC) scale.⁹ On the 5-point scale, a GMFC 3 represents children who achieved independent sitting by 4 years of age and ambulate with assistive devices, the aid of an adult, or both; a GMFC 4 represents children who ambulate minimally, even with assistance, and have poor sitting trunk control; and a GMFC 5 represents children who lack independent motor function even for basic antigravity postural control.

The magnetic resonance imaging (MRI) procedure used was modified from a procedure developed for adults.¹⁵ Subjects were immobilized from the waist down with BodyFIX (Medical Intelligence, Schwabmunchen, Germany), as previously described.^{16,17} Axial T1 weighted images (1-cm thick separated by 0.5 cm) were collected along the entire length of the non-dominant femur with a GE 1.5 Tesla magnetic resonance imager and a torso PA coil (TR = 750, TE = 14, FOV = 16, 1 NEX, 512 × 512 matrix).

Images at the level of the mid-third of the femur (ie, mid thigh) were analyzed on a personal computer with custom software developed with Interactive Data Language (Research Systems, Boulder, Colorado). The software automatically identifies images in the mid thigh on the basis of the number of images collected. AT was separated into intermuscular, subfascial, and subcutaneous subregions by using the raw images and the following semi-automated procedure. First, a line was traced directly over the deep fascia lata to separate subcutaneous AT from the intermuscular and subfascial AT. Second, a line was traced on the outside border of the femur to eliminate bone marrow from inclusion in the AT subregions. Third, a line was traced just inside the border of skeletal muscle to separate intermuscular AT from subfascial AT. In areas where adjacent muscles were separated, the line was drawn midway between the 2 muscle borders. The AT beneath the deep fascial layer was separated into intermuscular and subfascial subregions because the intermuscular sub-

region is more closely related to insulin sensitivity and has been shown to respond more strongly to intervention.² Fourth, images were filtered, and image segmentation was performed with a fuzzy clustering algorithm,¹⁸ in which pixels are categorized as AT, muscle, and background (or bone) on the basis of their signal intensity.

The cross-sectional area (CSA) of muscle, total AT, intermuscular AT, subfascial AT, and subcutaneous AT were determined for each image in the mid thigh. The average CSAs of all images in the mid thigh are reported. Because the image at the top of the mid thigh was partially shared by the upper thigh, and the image at the bottom of the mid thigh was partially shared by the lower thigh, the contribution of the soft tissue in these images to the overall average was weighted appropriately. The percentage of soft tissue in the mid thigh that was AT was calculated by using this equation:

$$[(\text{total AT mass in mid thigh} / (\text{total AT mass in the mid thigh} + \text{muscle mass in mid thigh})) \times 100].$$

Total AT mass in the mid thigh was determined by multiplying AT volume by 0.923 g/cc, the assumed density of AT.¹⁹ Muscle mass in the mid thigh was determined by multiplying muscle volume by 1.04 g/cc, the assumed density of muscle tissue.¹⁹ The volume of each tissue in the mid thigh was determined by multiplying its cross-sectional area by 1.5 in the middle images to account for the thickness of the image (1 cm) and the space between images (0.5 cm) and an appropriately weighted value for the image at the top of the mid thigh and the image at the bottom of the mid thigh. The ratios of intermuscular AT CSA, subfascial AT CSA, and subcutaneous AT CSA to muscle CSA in the mid thigh were created to account for differences in muscle size. To fully control for muscle CSA, the numerator was adjusted by subtracting the intercept of the regression of the numerator on the denominator, as suggested by Allison et al.²⁰ The ratios of intermuscular AT CSA and subfascial AT CSA to subcutaneous AT CSA in the mid thigh were created to reflect the regional distribution of AT. Intermuscular AT CSA, subfascial AT CSA, ratios of intermuscular and subfascial AT CSA to muscle CSA, and ratios of intermuscular and subfascial AT CSA to subcutaneous AT CSA in the mid thigh were viewed as markers of AT infiltration of skeletal muscle.

To assess the test-retest reliability of AT and muscle CSA in the mid thigh, 8 children (2 children with QCP and 6 typically developing children) 5 to 12 years of age were tested on the same day after repositioning or on different days within 2 weeks apart. The 2 image sets were not different in total AT ($62.4 \pm 12.3 \text{ cm}^2$ versus $61.8 \pm 12.3 \text{ cm}^2$), intermuscular AT ($2.5 \pm 1.6 \text{ cm}^2$ versus $2.4 \pm 1.5 \text{ cm}^2$), subfascial AT ($4.5 \pm 2.2 \text{ cm}^2$ versus $4.5 \pm 2.3 \text{ cm}^2$), subcutaneous AT ($55.4 \pm 9.3 \text{ cm}^2$ versus $54.9 \pm 9.3 \text{ cm}^2$), or muscle ($70.7 \pm 16.9 \text{ cm}^2$ versus $70.7 \pm 16.2 \text{ cm}^2$) CSA. The intraclass correlation coefficients were >0.99 for all repeat CSA measures.

Physical activity was estimated with an accelerometer-based activity monitor (Actical; MiniMitter, Sunriver, Ore-

gon) worn around the waist on the non-dominant side in typically developing children and on the more affected side in children with QCP. The Actical activity monitor was used because it was validated with a broad range of gross and fine motor physical activities.²¹ Monitors were worn continuously for 4 days (3 days during the week and 1 day on the weekend). Physical activity was divided into sedentary, light, moderate, and vigorous levels on the basis of activity counts registered every 15 seconds (sedentary <.01; light = 0.01-0.04; moderate = 0.04-0.10; vigorous >0.10 kcal/min/kg).²¹ Four days of data from an activity monitor has good reliability and validity.²²

Parents, with the aid of children (when able), recorded the child's dietary intake for 2 weekdays and 1 weekend day. To facilitate accurate quantification of foods, each subject and their parent received a list of serving size estimates on the basis of comparisons to everyday objects (eg, 3 oz of meat or poultry is approximately the size of a deck of cards).²³ Parents were contacted to clarify any uncertain foods or quantities. Total energy intake and the percent of energy intake from carbohydrate, fat, and protein were estimated from the diet records by using the USDA Food and Nutrient Database for Dietary Studies, 1.0.²⁴

Data were analyzed with SPSS software (version 15.0; SPSS, Chicago, Illinois). Variables were checked for normality with skewness (>2.00), kurtosis (>2.00), and the Shapiro-Wilk test. Group differences were determined with independent *t* tests when they were normally distributed and the Mann-Whitney *U* tests when they were not normally distributed. All data are presented as means plus or minus SD. To assess whether the degree of AT infiltration of skeletal muscle in the midthigh was associated with physical activity, relationships between the ratios of AT CSA in the different subregions of the midthigh to muscle CSA in the midthigh and total physical activity counts were evaluated with Pearson correlation analysis. When outliers were detected with Cook's *D* and standardized DfBeta scores >1 or when data were not normally distributed, data were transformed before correlations were assessed. The alpha level was set at 0.05. Sample size was determined using power charts from Cohen²⁵ and data from Leroy-Willig et al,⁸ in which total body intramuscular fat was much higher in boys with Duchenne muscular dystrophy than control children (Cohen's *d* = 3.0; *n* = 8/group). With a smaller effect size than observed in the Leroy-Willig et al study (Cohen's *d* = 1.5) and setting power at 0.9, it was determined that at least 10 subjects per group were needed to adequately address the primary aim of this study.

RESULTS

One girl and 1 boy in the QCP group could not complete testing because of fear of the MRI testing procedure. Characteristics of the subjects who completed the study are presented in Table I. There were 4 boys and 8 girls in each group. There were no group differences in age, Tanner stage, or BMI (*P* > .05). However, children with QCP had lower

Table I. Physical characteristics, diet, and physical activity in children with quadriplegic cerebral palsy and typically developing control subjects

	QCP (n = 12)	Controls (n = 12)
Age (years)	10.2 ± 2.5	10.3 ± 1.4
Tanner stage		
Pubic hair	1.3 ± 0.6	1.3 ± 0.6
Breast/testicular	1.3 ± 0.5	1.3 ± 0.6
Height (m)	1.26 ± 0.14*	1.42 ± 0.07
Height (%)	15 ± 16*	56 ± 20
Femur length (m)	0.33 ± 0.05*	0.39 ± 0.03
Body mass (kg)	28.2 ± 12.3*	35.3 ± 4.6
Body mass (%)	28 ± 37*	55 ± 24
BMI (kg/m ²)	17.0 ± 4.7	17.6 ± 2.0
BMI (%)	40 ± 38	52 ± 26
GMFC (level 3/4/5)	3/0/9	—
Diet†		
Carbohydrates (%)	48 ± 17	54 ± 6
Fat (%)	38 ± 19	32 ± 6
Protein (%)	14 ± 4	14 ± 3
Energy intake (kcal)	1691 ± 460	1926 ± 294
Physical activity		
Sedentary (counts/day)	4609 ± 1365	4773 ± 1010
Light (counts/day)	30 207 ± 19 544*	56 888 ± 25 233
Moderate (counts/day)	79 330 ± 121 934*	301 691 ± 87 564
Vigorous (counts/day)	4905 ± 12 033*	37 607 ± 25 378
Total (counts/day)	119 052 ± 148 857*	400 960 ± 101 564

Height (%), Height relative to age-based norms; body mass (%), body mass relative to age-based norms; BMI (%), BMI relative to age-based norms.

Values are means ± SD.

*Group difference, *P* < .05.

†*n* = 11 for children with QCP.

height (*P* < .01), height percentile (*P* < .001), femur length (*P* < .001), body mass (*P* = .04), and body mass percentile (*P* = .04) than control subjects. Height and body mass, but not BMI, were lower than the 50th percentiles for age in children with QCP (*P* < .05). Height, body mass, and BMI of control subjects were not different than the 50th percentiles for age (*P* > .05). Group comparisons of diet and physical activity are also presented in Table I. There were no group difference in the percentage of energy from carbohydrate, fat, and protein or total energy intake (all *P* > .05). There was no group difference in sedentary physical activity counts (*P* > .05); however, children with QCP had 47% fewer light (*P* = 0.02), 74% fewer moderate (*P* < .001), 87% fewer vigorous (*P* < .001), and 70% fewer total (*P* < .001) physical activity counts than control subjects.

Midthigh muscle and AT CSAs are reported in Table II. Children with QCP had 51% lower midthigh muscle CSA than control subjects (*P* < .001). Although there was no group difference in total AT CSA in the midthigh, the percentage of soft tissue in the midthigh that was AT was 42% higher in children with QCP than in control subjects (*P* < .001). In addition, children with QCP had a higher intermuscular AT CSA (2.3-fold; *P* = .01) and showed a

Table II. Muscle and adipose tissue cross-sectional area in the mid thigh of children with quadriplegic cerebral palsy and typically developing control subjects

	QCP (n = 12)	Controls (n = 12)
Muscle (cm ²)	35.5 ± 13.5*	72.0 ± 11.7
Total AT (cm ²)	53.8 ± 29.1	50.2 ± 15.8
% AT in soft tissue	57.8 ± 11.5*	40.6 ± 8.4
Intermuscular AT (cm ²)	3.5 ± 2.4*	1.5 ± 0.5
Subfascial AT (cm ²)	4.8 ± 3.4†	2.8 ± 0.8
Subcutaneous AT (cm ²)	45.5 ± 24.6	45.9 ± 14.8
Intermuscular AT/muscle	0.09 ± 0.05*	0.02 ± 0.01
Subfascial AT/muscle	0.12 ± 0.07*	0.04 ± 0.01
Subcutaneous AT/muscle	1.16 ± 0.57*	0.58 ± 0.23
Intermuscular AT/subcutaneous AT	0.08 ± 0.05*	0.03 ± 0.01
Subfascial AT/subcutaneous AT	0.11 ± 0.04*	0.06 ± 0.01

% AT in soft tissue, [Total AT mass / (AT mass + muscle mass)] × 100.

Values are means ± SD.

**P* < .05.

†*P* = .06.

trend for a higher subfascial AT CSA (1.7-fold; *P* = .06) in the mid thigh. Children with QCP also had a higher ratio of intermuscular AT CSA to muscle CSA (4.5-fold; *P* < .001), subfascial AT CSA to muscle CSA (3-fold; *d* = 2.10; *P* < .001), and subcutaneous AT CSA to muscle CSA (2-fold; *P* < .01) in the mid thigh. Moreover, children with QCP had higher ratios of intermuscular AT CSA to subcutaneous AT CSA (2.5-fold; *P* < .01) and subfascial AT CSA to subcutaneous AT CSA (1.8-fold; *P* < .01) in the mid thigh compared with control subjects. Representative images in Figure 1 (available at www.jpeds.com) show the greater AT infiltration of skeletal muscle in the mid thigh of a prepubertal girl with QCP versus a typically developing prepubertal girl.

When the relationship between physical activity and measures of AT infiltration of skeletal muscle were examined, an outlier was detected in the QCP group; therefore, total physical activity counts were transformed by taking their square root. Transformed total physical activity counts were significantly and inversely related to the ratio of intermuscular AT CSA to muscle CSA (*r* = −0.76; *P* < .01) and the ratio of subfascial AT CSA to muscle CSA (*r* = −0.63, *P* = .03), but not to the ratio of subcutaneous AT CSA to muscle CSA (*r* = −0.28, *P* = .38) in the mid thigh of children with QCP. None of the relationships were statistically significant in control subjects (*r* = −0.19, −0.37, and −0.39, respectively; *P* > .10). Scatter plots of transformed total physical activity counts versus the ratios of intermuscular AT CSA, subfascial AT CSA, and subcutaneous AT CSA to muscle CSA in the mid thigh are presented in Figure 2.

DISCUSSION

We assessed AT infiltration of skeletal muscle in children with QCP. The high degree of AT infiltration observed in children with QCP in this study is consistent with findings

Figure 2. Scatter plots of transformed physical activity versus **A**, the ratio of intermuscular adipose tissue (IMAT) CSA to muscle CSA in the mid thigh of children with QCP (*r* = −0.76; *P* < .01) and typically developing children (control subjects, *r* = −0.19; *P* = .56); **B**, the ratio of subfascial adipose tissue (SFAT) CSA to muscle CSA in the mid thigh of children with QCP (*r* = −0.63; *P* = .03) and control subjects (*r* = −0.37; *P* = 0.24); and **C**, the ratio of subcutaneous adipose tissue (SCAT) CSA to muscle CSA in the mid thigh of children with QCP (*r* = −0.28; *P* = .38) and control subjects (*r* = −0.39; *P* = .21).

in other groups with extremely limited physical activity and very low muscle mass.^{3,8} The finding of greater AT infiltration of skeletal muscle in children with QCP was not entirely expected, because in addition to their limited mobility, low muscle mass, and low energy expenditure, children with QCP

also show signs of under-nutrition, such as reduced body size.^{10,26,27} Consistent with these earlier studies,^{10,26,27} children with QCP in this study were shorter and lighter than typically developing children. Only recently has concern about excessive energy intake in children with QCP emerged, especially in children who are fed using a gastrostomy tube.²⁸ There are some indications that children with QCP in this study were consuming more energy than needed. For instance, despite a lower body mass in children with QCP than in control subjects, there was no group difference in energy intake. Furthermore, the 51% lower midthigh muscle CSA and 70% fewer activity counts in children with QCP than in control subjects suggests that children with QCP had much lower energy expenditure, which is consistent with earlier reports.^{4,27} However, because energy expenditure was not determined in this study and because there is evidence that parents of children with QCP overestimate their children's energy intake,²⁷ it cannot be determined whether excess energy intake contributed to the greater AT infiltration of skeletal muscle in children with QCP.

This study supports the notion that low physical activity is a major contributor to the high degree of AT infiltration of skeletal muscle in children with QCP. The inverse relationship between total physical activity counts and the ratios of intermuscular AT CSA and subfascial AT CSA to muscle CSA in the midthigh of children with QCP is consistent with a recent study by Manini et al⁷ in which adults who underwent lower limb suspension for 4 weeks experienced an increase in intermuscular AT in the thigh (14.5%) and calf (20%). The amount of subcutaneous AT in the calf and thigh did not change during the lower limb suspension period. Additional evidence suggesting physical activity drives the degree of AT infiltration of skeletal muscle is the recent finding that athletes with paraplegic spinal cord injury compared with non-athletes without spinal cord injury have higher intermuscular AT in their thighs, but similar amounts of intermuscular AT in their arms.²⁹ It is worth noting that physical activity was not significantly related to the ratios of intermuscular AT CSA to muscle CSA or subfascial AT CSA to muscle CSA in the midthigh in typically developing children in this study. The nonsignificant relationship may be attributed to their limited range of physical activity counts. Typically developing children were excluded from the study if they participated in organized physical activity >3 hours/week. It is also plausible that the relationship between physical activity and markers of AT infiltration of skeletal muscle is more easily detected within the lowest range of physical activity.

There are study limitations that should be considered. One limitation is that intramyocellular lipid in skeletal muscle was not assessed. A high level of intramyocellular lipid in skeletal muscle, like high levels of intermuscular and subfascial AT,² is associated with insulin resistance.³⁰ Although there is evidence that intramyocellular lipid content is elevated in individuals with neurological disorders,³¹ whether children with QCP exhibit a high proportion of intramyocellular lipid

is still unknown. A second study limitation is the absence of glucose tolerance and insulin sensitivity markers. Although higher proportions of intermuscular and subfascial AT are associated with poor glucose tolerance and insulin resistance in obese adults² and other groups with limited mobility,³ studies are needed to determine whether the relationships exist in children with QCP. A third study limitation is the unknown accuracy of accelerometer-based activity monitors in the assessment of physical activity in children with QCP. Although the monitors used in this study have been validated in a wide range of physical activities,²¹ additional studies that focus on QCP are needed. A final study limitation is that magnetic resonance images could not be obtained from all children with QCP. However, most subjects (12 of 14 with QCP and 12 of 12 typically developing children) were able to complete all testing successfully, suggesting that the application of MRI in the assessment of AT infiltration of skeletal muscle in children is promising.

The findings suggest that children with QCP have a greater AT infiltration of skeletal muscle than typically developing children, which is related to their very low level of physical activity. Whether this increased AT infiltration during childhood is associated with poor glucose tolerance, insulin resistance, and the high rate of death from diseases of the circulatory system observed in individuals with severe cerebral palsy requires further investigation. Moreover, studies that assess whether increased physical activity would reduce the degree of AT infiltration of skeletal muscle in children with QCP are warranted.

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Figure 1. A-C, The progressive separation of AT from magnetic resonance images of the midthigh of a prepubertal girl with QCP and D-F, a typically developing prepubertal girl. A and D contain subcutaneous, subfascial, and intermuscular AT; B and E contain only subfascial and intermuscular AT; and C and F contain only intermuscular AT.