# Changes in Brown Adipose Tissue and Muscle Development during Infancy

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**Objective** To examine the relationship between brown adipose tissue (BAT) and muscle development, two tissues that derive from a common cell lineage, during the first 6 months of postnatal life.

**Study design** Thirty healthy term infants (15 males and females) underwent whole-body magnetic resonance imaging examinations. Measurements of BAT in the supraclavicular area as well as measures of trunk musculature and subcutaneous adiposity were obtained at birth and at 6 months of age.

**Results** Paraspinous musculature and subcutaneous white adipose tissue (WAT) increased, and the proportion of BAT in the supraclavicular area decreased during infancy. Although measures of BAT did not correlate with paraspinous musculature through the first 6 months of life ($r = -0.35$; $P = .09$), BAT was a significant predictor of paraspinous musculature after adjusting for weight, body length, and WAT ($P = .002$); infants with the smallest decreases in BAT had the greatest gains in musculature. In contrast, changes in BAT did not predict increases in subcutaneous WAT ($P = .25$) during infancy, which were primarily determined by body weight.

**Conclusions** Changes in BAT are associated with muscle development but not WAT accumulation in healthy infants. Studies are needed to determine the mechanism(s) by which BAT could facilitate muscle growth, and the degree to which decreased muscle mass, such as in preterm and low birth weight infants, is related to a deficiency of BAT. *(J Pediatr 2016;173:116-21)*.

Infancy is a unique developmental stage associated with striking gains in muscle and bone, particularly in the axial skeleton, despite limited physical activity.[1] During the first months of life, healthy infants are relatively motionless and spend most of the day asleep. Because sensing and transducing mechanical loading information derived from muscle contractions is an essential anabolic signal for bone,[2] the maintenance of musculoskeletal integrity in the presence of decreased loading provides a strong case for other unidentified mechanisms.

Brown adipose tissue (BAT) is specialized to generate heat by dissipating chemical energy as a defense against cold. Previous anatomical studies indicate that BAT is established in fetuses within the fifth month of gestation.[3] At the time of birth, BAT abundance peaks as reflected by levels of UCP1, before declining over the next 6-9 months.[4,5] Data indicate that classical brown adipocytes develop during the prenatal stage from *myf-5* positive myoblast precursors,[6] and that BAT precursors show a gene signature that resembles skeletal muscle cells.[7] In addition, BAT mitochondria are most similar to their counterparts in skeletal muscle at both the transcriptional and protein levels.[8] Further support for a link between BAT and muscle come from clinical studies showing that the volume of BAT increases rapidly during late stages of puberty.[9] Hence, BAT is maximally recruited at birth and at sexual maturity, which precede marked gains in skeletal muscle during infancy and young adulthood.

Studying early postnatal development has been limited by animal models that fail to replicate human infancy, and the use of projection techniques that do not provide 3-dimensional assessments of the body composition of human infants.[10] Advances in magnetic resonance imaging (MRI) allow for fast, noninvasive assessments of both BAT and muscle development in newborns and infants without the need for sedation or ionizing radiation.[11,12] In this prospective longitudinal study, MRI techniques were used to examine the relations between changes in supraclavicular BAT and trunk muscle development during the first 6 months of life. Because animal investigations have shown that a reduced amount of BAT leads to obesity,[13,14] and human studies have reported an inverse relationship between BAT and white adipose tissue (WAT),[15,16] a secondary goal was to examine the relations between BAT and subcutaneous WAT during early postnatal life.

## Methods

The study population was composed of 30 white singleton term infants (15 males and 15 females; aged 2-7 days), who were recruited from The Institute for Maternal-Fetal Health–Children's Hospital Los Angeles between September

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| BAT | Brown adipose tissue |
| FF | Fat fraction |
| MRI | Magnetic resonance imaging |
| WAT | White adipose tissue |

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2014 and January 2015. The Institutional Review Board for clinical investigations at Children's Hospital Los Angeles approved these studies, which were compliant with the Health Insurance Portability and Accountability Act. Written informed consent was obtained from the parent(s) of all subjects.

Only neonates who were the product of a term pregnancy (37-42 weeks) with a birth weight, length, and head circumference between the 10th and 90th percentiles (according to World Health Organization growth charts), a 1-minute Apgar score of ≥7, a 5-minute Apgar score of ≥8, and no history of cardiac, respiratory, gastrointestinal, or other systemic disease were included in this study. Infants born to mothers who were not planning to breastfeed for ≥6 months and with a history of diabetes or gestational diabetes mellitus or the consumption of tobacco, alcohol, or illegal drugs were excluded from this study. Eligible participants were asked to return for follow-up examinations, including MRI studies of body composition, at 6-7 months of age. A dietary questionnaire was completed by the infant's mother at the follow-up visit, indicating how many days in the past week, did the baby consume breast milk, plain water, nonmilk liquids, nonhuman milk, semisolids/solids, fermented milk/cereal, or anything else.[17] Infants were categorized at each visit as one of the following: (1) stopped breastfeeding; (2) low breastfeeding (mixed fed 5-7 days); (3) high breastfeeding (mixed fed 1-4 days); or (4) exclusive breastfeeding.

All baseline and follow-up studies were performed without the use of general anesthesia and/or sedatives. Parents were instructed to keep the infant awake as long as possible before the study and to plan a feeding (nursing or bottle) immediately before the scan. Then, the infant was swaddled in cloth blankets, given protective ear muffs, and monitored with a pulse oximetry system. All subjects were examined with a 3.0 Tesla whole-body MRI scanner (Philips Healthcare, Cleveland, Ohio) with a standard 16-channel torso array coil. Sagittal, coronal, and axial T2-weighted images were acquired using a single shot fast spin-echo sequence with 3-mm-thick slices without any gap and 1-mm in-plane resolution to evaluate for muscle and subcutaneous adiposity. The repetition time ranged from 562-644 ms and the echo time ranged from 102-120 ms. Acquisition time was between 27-63 seconds. Additionally, as described, we used a low flip angle (3°) 6-echo proton density-weighted chemical shift-encoded water-fat pulse sequence to evaluate for BAT.[18]

Image analysis was performed with SliceOmatic image segmentation software (Tomovision, Inc, Magog, Canada). Paraspinous musculature cross-sectional area was defined as the mean of the cross-sectional areas of the erector muscles of the spine and psoas major muscle at the 5 lumbar vertebrae. Measures of subcutaneous WAT were obtained at the same locations. Because the resolution of clinical imaging modalities does not allow for the identification of cellular structures, we used MRI measures of fat fraction (FF) as surrogate measures of BAT. The intrinsic morphological differences between WAT (ie, greater fat content) and BAT (ie, greater water content) give rise to unique signatures that can reliably be detected and quantified by chemical shift water-fat MRI using FF = fat/(fat + water). A high FF is indicative of WAT, and lower FF values denote a greater presence of BAT.[18] For the purpose of this study, mean FF values were calculated in the supraclavicular depot, which was defined as adipose tissue bound medially by the sternocleidomastoid muscle, inferiorly by the clavicle, and posteriorly by the trapezius muscle (**Figure 1**). Based on a prior study of ex vivo samples, BAT was correctly identified 99% of the time with a WAT false-positive rate of <10% at the upper threshold of 60% FF; at the 20% FF lower threshold, BAT was correctly identified 99.9% of the time.[12]

### Statistical Analyses

Paired *t* tests were used to determine whether continuous variables differed between baseline and 6-months and independent *t* tests to determine whether continuous variables differed between sexes. If *t* test assumptions were not met, Wilcoxon signed-rank test and Mann-Whitney tests were used in lieu of paired and independent *t* tests. The Fisher exact test determined the influence of sex and feeding practices at the 6-month follow-up. A within-subjects Pearson correlation, which accounts for the lack of independence among repeated measurements by removing the variation between subjects, was calculated to correlate BAT with paraspinous musculature and WAT. Mixed effects regression analysis was used to account for the small number of repeated measures among multiple subjects and to determine predictors of paraspinous musculature, WAT, and supraclavicular FF while adjusting for appropriate covariates with the random effects occurring at the patient level with an identify covariance structure. To assess whether a variable significantly added to the regression model, likelihood ratio tests, Akaike and Schwarz Bayesian information criteria were compared between unadjusted and adjusted models. Continuous variables are presented as mean values ± SD, and categorical variables as frequency (percent of sample). Regression variables are presented as coefficient (95% CI). A 2-sided *P* of <.05 was considered significant. Data analysis was performed using Stata (StataCorp, College Station, Texas).

## Results

The age, anthropometric characteristics, and MRI measures of body composition at baseline and follow-up at 6 months for boys and girls, and all subjects are described in **Table I**. With the exception of head circumference values, which were greater in boys, the sexes were monomorphic with regard to all anthropometric measures at birth, and neither measures of subcutaneous WAT, paraspinous musculature, or FF in the supraclavicular area differed among newborn boys and girls.

Values for all anthropometric traits increased during infancy (**Table I**). Regardless of sex, paraspinous musculature and subcutaneous WAT increased during infancy, and the