Review

Relevance of brown adipose tissue in infancy and adolescence

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Brown adipose tissue (BAT) was thought to disappear after infancy. Recent findings of BAT in patients undergoing positron emission tomography/computed tomography (PET/CT) have renewed the interest in deciphering the relevance of this tissue in humans. Available data suggest that BAT is more prevalent in children than in adults and that its activation during adolescence is associated with significantly lower gains in weight and adiposity. Data also show that pediatric patients with metabolically active BAT on PET/CT examinations have significantly greater muscle volume than patients without identifiable BAT. Both the activity and the amount of BAT increase during puberty. The magnitude of the increase is higher in boys as compared with girls and is closely related to gains in muscle volume. Hence, concurrent with the gains in skeletal muscle during infancy and puberty, all infants and adolescents accumulate large amounts of BAT. These observations are consistent with *in vitro* investigations suggesting close interactions between brown adipocytes, white adipocytes, and myocytes. In this review, we discuss the potential role of this tissue in regulating weight and musculoskeletal development in children.

INTRODUCTION

It has been nearly five centuries since Konrad Gessner described a tissue that was "neither fat, nor flesh, but something in between (1)," five decades since Robert E. Smith showed the capacity of brown adipose tissue (BAT) to dissipate energy as heat (2), and 5 y since the ranks of obese individuals overtook the number of malnourished individuals in the world (3), but whether BAT has any relevance to human health and disease beyond helping to maintain normal body temperature in newborns remains unknown. The belief that BAT involutes soon after birth and the lack of techniques to adequately measure BAT in humans have limited our understanding of the relevance of this tissue.

New data showing that some adults and most children have BAT, coupled with the development of imaging techniques that provide reliable BAT measurements, has renewed the interest in deciphering the physiology of this tissue (4,5). In this review, we discuss recent advances in our understanding of the potential role of this tissue in regulating weight and musculoskeletal development in children and adolescents.

ANATOMY, PHYSIOLOGY, AND PATHOLOGY OF BAT

The adipose organ is a complex endocrine system, composed of white and brown fat. White adipose tissue (WAT) serves as the primary site of energy storage, storing triglycerides within individual adipocytes, whereas BAT stores little fat, burning it instead to produce heat and regulate body temperature (6,7). As compared with WAT, BAT is highly vascularized and innervated by the sympathetic nervous system. Moreover, white adipocytes are spherical and unilocular, whereas the brown adipocyte is usually smaller and characterized by multilocular lipid droplets and an abundance of mitochondria expressing uncoupling protein-1 (UCP1) (7). In humans, BAT is recognized primarily in the cervical–supraclavicular area, where it is present across a wide range of ages (4,5,8,9). To date, the only known function of BAT is to dissipate energy through the uncoupling of oxidative respiration from the production of adenosine triphosphate, an action regulated by UCP1 (7,10,11). This type of heat production is known as nonshivering thermogenesis.

In a thermoneutral state, the activity of UCP1 in the mitochondrial membrane is inhibited by adenosine triphosphate in the cytoplasm. During cold acclimation, sympathetic stimulation triggers the proliferation and differentiation of precursor cells toward the development of BAT (12). Mitochondrial biogenesis and increased synthesis of UCP1 are hallmarks of the thermogenic recruitment process. The release of norepinephrine, associated with a cold environment, interacts with β-adrenergic receptors in the cell membrane of the brown adipocyte, leading to the hydrolysis of triglycerides and the production of free fatty acids (13). The increase in free fatty acids in the cytoplasm overcomes adenosine triphosphate inhibition by interacting with UCP1, leading to its activation. UCP1 increases proton leakage across the inner membrane of brown adipocyte mitochondria, dissipating energy in the form of heat rather than adenosine triphosphate (12).

Besides UCP1, four additional uncoupling proteins are expressed by the human genome. Although UCP1 is exclusive to BAT, UCP2 is expressed ubiquitously, UCP3 is mainly expressed in skeletal muscle, and UCP4 and UCP5 are expressed in the brain (14). The physiological functions of these proteins are not well understood but could have profound significance in our understanding of conditions such as diabetes, obesity, thyroid disease, and aging. Thyroid hormone, an important regulator of

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energy expenditure, is not only necessary for the full expression of UCP1 but also for UCP3 expression and UCP3-mediated uncoupling in skeletal muscle mitochondria of rodents (14).

There are two rare pathological disorders closely related to BAT. Hibernomas are benign tumors arising from brown adipocytes; hibernomas have primarily been described in the neck, axilla, thoracic regions, and retroperitoneum most commonly in young adults (15). Subcutaneous fat necrosis of the newborn is another clinical condition, often associated with asphyxia or hypothermia, that pathologically is characterized by focal areas of fat necrosis that are infiltrated by macrophages and brown adipocytes at several stages of degeneration (16).

RECENT ADVANCES IN PEDIATRIC BAT RESEARCH

Until recently, the accumulated information on the thermogenic effect of BAT was derived from studies on rodents and hibernating mammals, with little awareness of its potential relevance to humans. Although first described in neonates back in 1902 (17), BAT only began to take on a life of its own a century later with the introduction of positron emission tomography/computed tomography (PET/CT). Today, a large body of evidence indicates that metabolically active BAT is present in a significant number of patients with cancer undergoing PET/CT examinations (4,5,8,10). However, there is a strikingly higher prevalence of BAT in pediatric PET/CT examinations (ranging from 31 to 77%) (4,18–22) as compared with the prevalence in adults (5), ~1 in 2 children vs. 1 in 20 adults.

The depiction of BAT by PET/CT in children, as in adults, is related to environmental temperature and season. BAT activity is observed in most studies during cold exposure (4,23), consistent with histological evidence indicating that BAT is universally present in children and adults (24,25). The percentage of PET/CT studies with metabolically active BAT is also higher in the winter months. This is true even in children living in warmer climates and when studies are obtained under thermoneutral conditions (4,18). Indeed, it is becoming increasingly apparent that photoperiod and day length are strong determinants of BAT activity, independent of environmental temperature (6,26).

Visualization of BAT in children is also dependent on disease status, weight, body composition, and the degree of sexual development (4,18,27,28). Although excessive calorie intake has also been suggested to stimulate BAT activity in mice, whether diet induces BAT activation and thermogenesis in humans is the subject of considerable discussion (29). However, UCP1 polymorphisms have been reported to influence postprandial thermogenesis after a high-fat meal in healthy boys (30).

A recent study in children with lymphoma found that whereas only ~10% of the PET/CT examinations at diagnosis exhibited BAT, ~80% of the follow-up examinations displayed BAT when the patients were in remission (27). Although the mechanism responsible for the suppression of BAT activity is unknown, patients with lymphomas have high circulating levels of tumor necrosis factor-α (31), a pluripotent cytokine reported to elicit apoptotic degeneration of brown adipocytes (32). This cytotoxic effect is known to be mediated by the p55 tumor necrosis factor-α receptor subtype, and its deletion has been shown to increase thermogenesis with an associated increase of UCP1 expression in BAT (33).

In contrast, the visualization of metabolically active BAT in adult patients with cancer is thought to be independent of disease status (34). Unfortunately, the low BAT prevalence, long treatment courses, and relatively poor survival rates greatly hinder longitudinal assessments of BAT in adult populations.

Association of BAT With Weight and Measures of White Adiposity in Children

Data from animal studies indicate that a reduced amount or function of BAT leads to obesity, insulin resistance, and dyslipidemia, whereas an increased amount or function protects against weight gain and its comorbidities (35–38). Studies in humans also suggest that WAT and BAT are inversely related (5,6,8,11,34). Lean patients exhibit greater BAT activity than obese subjects, and most studies in adult patients report a negative relation between body mass and/or body fat and the degree of metabolically active BAT (5,6,9). Other studies, however, found no relation between BAT and body mass (39,40) or measures of adiposity (40,41). Similar discrepancies are reported in pediatric cross-sectional studies; whereas one observed an inverse relationship between BMI and BAT activity (20), others found no significant differences in the weight, BMI, or measures of subcutaneous adiposity between children with and without functioning BAT (18,23).

The activation of BAT in children has been shown to be related to changes in weight and adiposity (28). Pediatric patients with cancer with no visualization of BAT at diagnoses, but with evidence of BAT activity at follow-up PET/CT studies, gained significantly less weight and subcutaneous and visceral adiposity than those who remained without BAT activity when disease-free. On average, increases in weight and subcutaneous fat were three times greater, and those in visceral fat six times greater, in children who did not demonstrate any BAT activity as compared with children who had metabolically active BAT (28).

The results of a recent study based on magnetic resonance imaging provide additional evidence that overweight/obese children have significantly less total (functional and nonfunctional) BAT in the supraclavicular area than lean, healthy children (H.H. Hu, L. Yin, P.C. Aggabao, T.G. Perkins, J.M. Chia, V. Gilsanz, unpublished data) (**Figure 1**). Moreover, there appears to be strong inverse relation between magnetic resonance imaging measures of BAT and weight or BMI percentile in overweight/obese children. Of note, the strength of this negative relation is similar to the strength of the positive correlation between values of WAT and these anthropometric measures.

Although BAT activation could decrease WAT as a result of increased energy consumption (8), it is also possible that WAT suppresses BAT function. The white adipocyte is known to produce cytokines and chemokines, such as tumor necrosis factor-α, interleukin-6, and monocyte chemoattractant protein-1, that induce inflammation and could potentially have cytotoxic effects on BAT (42,43). In addition, an increase in BAT could directly lead to an increase in muscle function and energy expenditure, which, in turn, leads to a decrease in

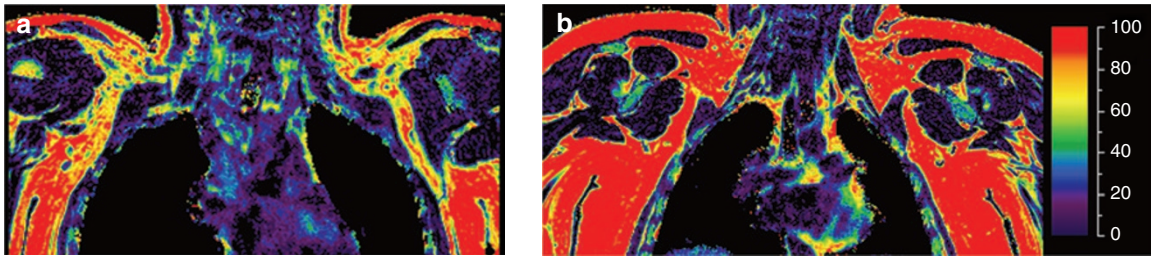

Figure 1. Presence of brown adipose tissue and BMI percentile. Coronal magnetic resonance views of (**a**) a healthy 11-y-old boy with a BMI in the 79th percentile depicting both white and brown fat (represented by a yellow/green fat fraction) and of (**b**) an obese 11-y-old boy with a BMI in the 98th percentile showing only white fat (represented by a red fat fraction). The fat fraction (%) scale is represented on the right.

adiposity. Studies are needed to establish the direction of causality between BAT activity and WAT accumulation and the degree to which this relationship is mediated by muscle.

Association of BAT With Muscle Development in Children

Recent data show that pediatric patients with metabolically active BAT on PET/CT examinations have significantly greater muscle volume than patients without identifiable BAT (18). On average, boys and girls who exhibit BAT have ~33–50% greater muscle volume than patients who do not exhibit BAT (18). This clinical observation is consistent with information that brown adipocytes and myocytes share many features, including an abundance of mitochondria, energy expenditure via oxidative phosphorylation, and sympathetically mediated adaptive thermogenesis (38,44–46). They also express myogenic factors, such as myf5, and may derive from a common lineage in the paraxial mesoderm (46,47). Further support for a muscle–BAT link comes from a landmark investigation indicating that exercise-induced gains in muscle lead to an increase in the amount of cells with a brown fat phenotype (48). This study identifies a new hormone, irisin, as an exercise-induced myokine that allegedly activates the induction of brown adipocytes in WAT depots, a process known as white fat "browning."

Skeletal musculature increases substantially during puberty. Gains in musculature associated with sexual development closely equal the growth of all other organs, systems, and tissues combined. Concurrently, a higher prevalence and large amounts of BAT are also present during adolescence. Although <20% of PET/CT examinations in prepubertal girls or boys exhibit metabolically active BAT, >75% of such studies in pubertal teenagers display BAT uptake (4). In addition, the volume of BAT increases during puberty in both sexes (**Figure 2**). The magnitude of the increase is substantially greater during the late stages of sexual development, higher in boys as compared with girls, and closely related to gains in muscle volume (18). Although the reasons for the pubertal increase in BAT are unknown, data suggest that sex steroids and growth hormone have a marked effect on BAT activity (49,50). Of note, past postmortem studies suggest UCP1 activity to be higher in teenagers as compared with neonates (51).

Association of BAT With Skeletal Development in Children

Two recent clinical studies suggest that BAT might be involved in the regulation of bone mass in humans. The first study, in young women with anorexia nervosa, reported a positive relation

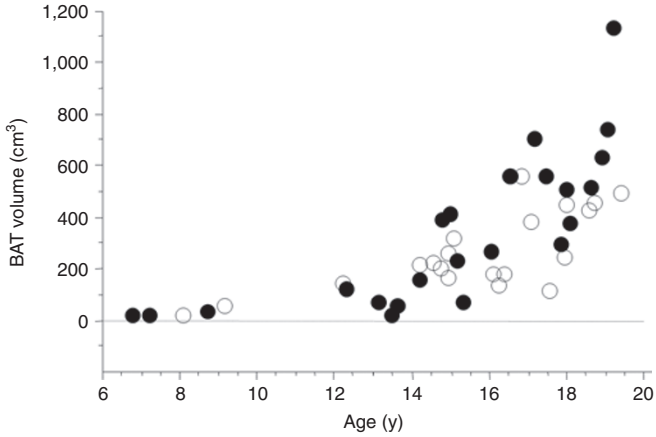

Figure 2. Gains in brown adipose tissue (BAT) during adolescence. Regardless of sex, BAT volume increased with age in boys (black) and girls (white) ($r = 0.77$ for boys and $r = 0.72$ for girls; both $P < 0.001$). Adapted from ref. 4, copyright 2012, with permission from Elsevier.

between BAT and bone density in the axial skeleton, which was independent of disease status and body mass (52). The second study, in children, found the volume of BAT to be positively related to the amount of bone in the appendicular skeleton, a relation that was also independent of known major determinants of bone acquisition, such as height, weight, and gender (53).

The reason(s) for the association between BAT and bone mass are yet to be defined. Available data, however, support a link between BAT and bone formation. The retinoblastoma protein was recently identified as a mesenchymal cell–fate regulator that controlled differentiation into either the brown adipocyte or the osteoblast (54). Several reports have found this key regulator to be capable of both inhibiting adipogenic differentiation and promoting osteoblast maturation (54,55). Studies in animal models also suggest that BAT may be involved in regulating osteoblastogenesis. Heterotrophic ossification modeled by the bone morphogenic protein-2 is known to induce the accumulation of brown adipocytes and subsequently trigger chondrocyte development and bone formation (56). Moreover, mice lacking functional BAT have very low bone mass, reduced osteoblast activity, and increased bone resorption (57).

Regardless of the mechanism by which BAT influences skeletal growth, maintaining optimal bone mass depends on sensing and transducing mechanical loading information derived from muscle contractions (58). Therefore, the possibility exists

for muscle to mediate the relationship between BAT and bone development (53). Support for the notion that BAT is crucial to the maintenance of musculoskeletal integrity comes from two physiologic situations characterized by an abundance of BAT in which decreased skeletal loading and locomotion do not result in muscle or bone loss. Large hibernating mammals are remarkably capable of maintaining their muscle and bone mass despite losing a third of their weight and remaining immobile over a period of nearly 7 mo (58,59). Similarly, infancy is a developmental stage associated with rapid increases in muscle and bone mass despite the lack of significant skeletal loading. Studies are needed to determine the degree to which BAT contributes to the maintenance of muscle function in the absence of mechanical strains associated with loading or locomotion.

FUTURE DIRECTIONS

To date, most studies on the molecular regulation of BAT have been conducted in animal models; these suggest that at least two types of brown adipocytes from distinct lineages exist: those of myoblast origin and those of adipocyte origin (45). Classic brown adipocytes (i.e., "preexisting" brown adipocytes) that reside in the interscapular BAT depot form during the prenatal stage from myoblastic-like myf5-positive precursors and have a gene profile similar to that of skeletal muscle (46) (**Figure 3a**). These myf5-positive cells differentiate into brown adipocytes through the action of the transcriptional regulators PRDM16, peroxisome proliferator-activated receptor-γ, and/or CCAAT/enhancer binding protein-β. In addition, pockets of a second, distinct type of brown adipocyte are found sporadically in the WAT of adult animals that have been exposed to chronic cold or to peroxisome proliferator-activated receptor-γ agonists. Although these inducible brown adipocytes, also known as brown-in-white cells, possess many of the biochemical and morphological characteristics of brown adipocytes, including the presence of multilocular lipid droplets and UCP1 expression (60), they arise from a non-myf5 cell lineage (**Figure 3b**).

There should ultimately be increased scope for studies decoding the transcriptional control of human brown fat development. This is especially pertinent because the gene profile of specialized tissues, such as BAT, has a very different molecular signature in humans as compared with mice (61). Recent data report differences in the response of UCP1 mRNA to hormonal stimulation even between rat and mouse brown adipocytes (62). Emerging questions that must be addressed regarding the biological significance of the two types of brown adipocytes in humans include: What are the molecular or functional differences between the two types of brown adipocytes? Are the molecular signatures of these cells in humans closer to that of myocytes or to that of white adipocytes? How relevant is the inducible brown adipocyte for the control of energy homeostasis as well as for obesity and metabolic disease? Of note are data indicating that preadipocytes isolated from supraclavicular fat in humans aged 35–64 y are capable of differentiating into brown adipocytes *in vitro*, regardless of PET status (63). Hence, molecular pathways of brown fat development should be intact and can be reactivated in adult humans. In this regard, synthetic peroxisome proliferator-activated receptor-γ ligands such as thiazolidinedione, widely used in drugs to treat type 2 diabetes, can direct white preadipocytes into mature brown adipocytes (64–66); this peroxisome proliferator-activated receptor-γ ligand–induced browning effect is reported to be mediated through stabilization of the PRDM16 protein (67).

Recently, we examined the relevance of brown-in-white cells in humans by analyzing the molecular signature of human BAT isolated from infants and adolescents. To our surprise, BAT in children predominantly expressed brown-in-white cell–selective genes rather than preexisting brown fat–selective genes. These data indicate that human BAT possesses molecular signatures that resemble those of beige cells (68).

Obesity has become the leading cause of preventable death. Because lean children appear to have greater BAT activity than obese subjects, there is considerable interest in defining the mechanisms responsible for relations between BAT and body composition. As it also seems that BAT and muscle mass are positively related and higher muscle mass is associated with better insulin sensitivity and lower risk of prediabetes (69), it is imperative that we examine the influence of BAT on metabolic health. Specifically, there is a need to establish whether a deficiency in BAT in early postnatal life permanently increases the risk of obesity and its comorbidities throughout life.

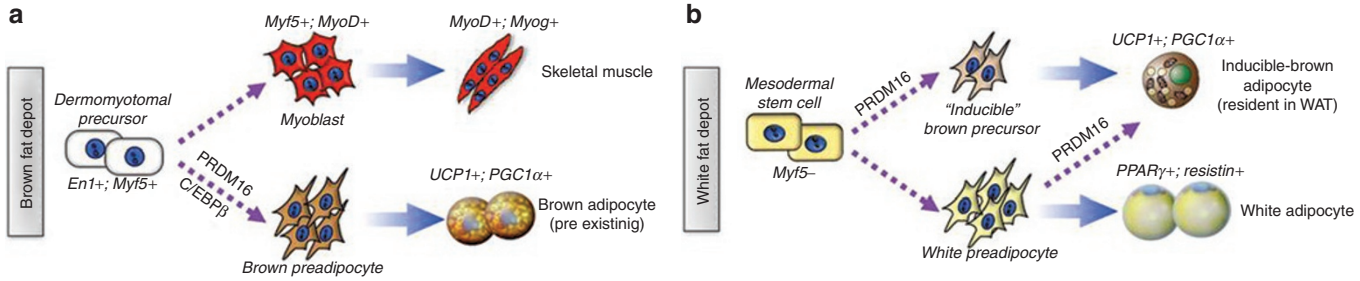

Figure 3. Differential developmental origins of brown and white adipocytes. (**a**) Brown adipose tissue (BAT) and (**b**) white adipose tissue (WAT) have separate developmental origins in the embryo. (**a**) BAT and skeletal muscle originate during the prenatal stage from precursors in the dermomyotome that express engrailed-1 (En1) and myf5. PRDM16, peroxisome proliferator-activated receptor-γ (PPARγ), PGC-1α, and uncoupling protein-1 are functional markers of brown adipose cells in the developmental, homogenous deposits of BAT. (**b**) The embryonic stem cells of the white adipose lineage remain to be well defined. The "inducible" brown adipocytes in WAT develop in response to cold, β-adrenergic stimulation, or PPARγ agonists. These cells may be derived from myf5-negative (myf5–) brown precursors or from directed differentiation from white preadipocytes or from mature white adipocytes. PGC, peroxisome proliferator-activated receptor γ coactivator. Adapted from ref. 45, copyright 2010, with permission from Elsevier.

Of equal importance is to determine how the transcriptional and epigenetic regulatory networks that govern human brown adipocytes respond to early stages of human postnatal growth. Postmortem studies indicate that BAT is established in fetuses within the fifth month of gestation (70). At the time of birth, BAT abundance peaks as reflected by the levels of UCP1, before declining over the next 9 mo (51). Studies are needed to examine the degree to which BAT accounts for phenotypic differences among infants and the degree to which BAT is influenced by maternal health and diet, gestational age, birth weight, and feeding practice. For example, current data show that as compared with formula-fed infants, breast-fed infants are leaner and grow more slowly (52). Not only do breast-fed infants grow at different rates during infancy, but breastfeeding appears to have a profound long-term influence on metabolism and disease risk later in life (71). The notion that BAT accounts for the leaner phenotype of breast-fed infants is supported by observations that leptin, ghrelin, adiponectin, resistin, and obestatin, all hormones involved in energy balance regulation, are identified in breast milk (72). Indeed, both leptin and adiponectin concentrations in breast milk have recently been found to influence UCP1 expression in BAT and to negatively correlate with infant body weight (73).

Given that BAT is a highly dynamic and elusive tissue that can exist in a variety of states depending on a wide spectrum of environmental factors, other noninvasive approaches beyond PET/CT are needed to assess the relevance of this tissue in humans. Thermal imaging is a rapid, nonionizing, and acceptable technique that can reliably quantify thermogenesis within the supraclavicular region in humans (74,75). Increases in BAT activity are closely related to a rise in depot temperature. However, the accuracy of these measures decreases in obese subjects because they are influenced by the amount of subcutaneous tissue in the supraclavicular fossa (76).

Magnetic resonance imaging developments will probably be a major driving force in deciphering the relevance of BAT in children. On the basis of the cytological differences in lipid content and degree of vascularization between BAT and WAT, fast magnetic resonance techniques are being developed that provide reliable BAT measures that can be applied even to infants without the need for sedation (77,78) (**Figure 4**). It should be noted that BAT depots contain a mixture of multilocular brown adipocytes interspersed within unilocular white adipocytes and that no imaging modality currently has sufficient resolution to localize microscopic deposits of brown adipocytes within a mixed cell population.

Finally, emerging evidence indicates circadian rhythms in BAT activity (79). Data in mice using PET/CT show a diurnal rhythm in glucose uptake by BAT (80). Studies are needed to establish the rhythms in clock gene expression in BAT in humans and whether the activity of this tissue increases during the night. Given the close association among exercise-induced muscle function, the release of irisin, and the formation of brown fat–like adipocytes (48), it is essential to investigate the possible correlate to complete the cycle: Does sleep-induced BAT activity promote structural and metabolic changes in skeletal muscle? And which brown adipokine(s) governs this adaptive response? Only then will we be in a position to begin to understand the mechanism(s) by which musculoskeletal development progresses during periods of inactivity.

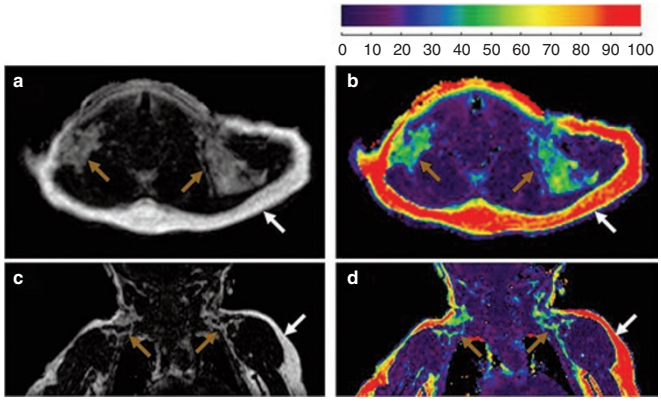

Figure 4. Imaging characteristics of brown and white adipose tissue in infancy. Axial (**a**,**b**) and coronal (**c**,**d**) magnetic resonance views of a 4-mo infant depicting both white fat (white arrow) and brown fat (brown arrow) at the level of the thoracic inlet. (**a**,**c**) As compared with white fat, brown fat is hypointense/darker in the fat images, and (**b**,**d**) has a lower fat fraction (green vs. red) in the coregistered fraction (fat/fat+water) color-scaled images. Adapted from ref. 68.

CONCLUSION

Brown fat is known to have been present in mammals over 150 million years ago and was considered an evolutionary advantage solely due to its unique ability to enhance survival in cold environments (7,81). However, it is becoming increasingly clear that this tissue may have greater relevance to human health. The main areas of progress in BAT research during the last decade have been (i) the general acceptance that this tissue is present in humans of all ages and is especially abundant during adolescence; (ii) the recognition that BAT may not only dissipate energy in the form of heat but may also be a key determinant of weight and musculoskeletal development during childhood; and (iii) insights into the complex transcriptional controls of brown fat development. Although much more work is needed, it is tempting to think that our challenge for the next decade lies in delineating the molecular regulation of BAT in humans and the crosstalk between brown and white adipocytes and myocytes. Defining the implications that BAT has for early human growth and how it influences health as we age is a most attractive and promising field of research.

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