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Brown adipose tissue in young adults who were born preterm or small for gestational age

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Abstract

Background: Brown adipose tissue (BAT) is present and functions to dissipate energy as heat in young adults and can be assessed using magnetic resonance imaging (MRI) to estimate the voxel fat fraction, i.e. proton density fat fraction (PDFF). It is hypothesized that subjects born preterm or small for gestational age (SGA) may exhibit disrupted BAT formation coupled to metabolic factors. Our purpose was to assess the presence of BAT in young adults born extremely preterm or SGA in comparison with controls.

Methods: We studied 30 healthy subjects (median age, 21 years): 10 born extremely preterm, 10 full term but SGA and 10 full term with a normal birth weight (controls). We utilized an MRI technique combining multiple scans to enable smaller echo spacing and an advanced fat-water separation method applying graph cuts to estimate B_0 inhomogeneity. We measured supraclavicular/cervical PDFF, $R2^*$, fat volume, insulin-like growth factor 1, glucagon, thyroid stimulating hormone and the BAT-associated hormones fibroblast growth factor 21 and irisin.

Results: The groups did not significantly differ in supraclavicular/cervical PDFF, $R2^*$, fat volume or hormone levels. The mean supraclavicular/cervical PDFF was equivalent between the groups (range 75–77%).

Conclusions: Young adults born extremely preterm or SGA show BAT development similar to those born full term at a normal birth weight. Thus, the increased risk of cardiovascular and metabolic disorders in these groups is not due to the absence of BAT, although our results do not exclude possible BAT involvement in this scenario. Larger studies are needed to understand these relationships.

Keywords: adults and pediatrics; endocrine disorders; magnetic resonance imaging.

Introduction

Epidemiological and clinical studies indicate that preterm and small-for-gestational age (SGA) infants have an increased risk of developing cardiovascular and metabolic disorders in childhood and adulthood [1–3]. Some authors have hypothesized that the underlying cause is disrupted brown adipose tissue (BAT) formation coupled to insulin-like growth factor 1 (IGF-I) [4]. It is thought that BAT and white adipose tissue (WAT) formation during fetal development occurs via differentiation of preadipocytes to adipocytes, with insulin and IGF-I playing major roles in the transition [4]. Knock-out mice lacking IGF-I and insulin receptors in their adipose tissue show almost no BAT, and are resistant to obesity and glucose intolerance induced by a high-fat diet [4]. Fetal BAT cells are a target for IGF-I, and the IGF-I receptor is an important modulator of BAT apoptosis and survival during fetal development [5]. IGF-I levels during the first months of life have been shown to predict and correlate with body composition at 2 years in preterm infants born SGA [6]. Certain studies indicate higher IGF-I levels in prepubertal children born SGA at term [7] or very preterm [8] compared with full-term appropriate-size-for-gestational-age controls. Furthermore, studies indicate that low birth weight, due to prematurity or SGA birth, is associated with altered adipose tissue distribution and increased trunk fat mass in adolescence [9–11].

Although it was previously thought that BAT in humans was limited to newborns, studies now show that BAT is present and functionally active in adults, primarily

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in the supraclavicular and lower cervical regions [12–14]. A key difference between BAT and WAT is that BAT can dissipate energy as heat through uncoupled respiration mediated by uncoupling protein 1 (UCP-1). BAT imaging has typically been performed using ^{18}F -fluoro-D-glucose (^{18}F -FDG) positron emission tomography (PET) computed tomography; however, this procedure exposes the subjects to high levels of ionizing radiation. Non-ionizing radiation methods, such as magnetic resonance imaging (MRI), have been developed and are preferable, especially in young adults and in longitudinal studies [15, 16]. By simultaneously comparing ^{18}F -FDG PET and MRI, it has been shown that BAT can reliably be measured using MRI without cold exposure [17]. With the proton density fat fraction (PDFF) method, the voxel fat/water fraction is measured using a multi-echo gradient-echo MRI sequence and a chemical shift-based fat-water separation technique, which also involves $R2^*$ determination. BAT shows a lower PDFF than WAT because BAT contains multivacuolar adipocytes mixed with univacuolar adipocytes [18], while WAT contains only univacuolar large adipocytes [19]. Additionally, BAT is highly vascularized and BAT cells contain large quantities of iron-rich mitochondria [19]; therefore, BAT has a higher $R2^*$ than WAT [20]. Franz et al. [21] used MRI for BAT evaluation in a cohort study. They reported that elderly subjects had no BAT in the supraclavicular/cervical region, which correlated with a fat fraction of approximately 90% (similar to WAT). On the other hand, children and adolescents showed the presence of BAT in the supraclavicular/cervical region, with a fat fraction of approximately 70%. Their study used signal fat-fraction for BAT estimation, but PDFF is even more accurate [21].

The present study aimed to obtain BAT PDFF data from healthy young adults and compare these findings to results in adults who were born preterm or SGA who hypothetically could exhibit disrupted BAT formation. The study was designed as a small-scale study, i.e. a pilot study, and therefore no power calculation was performed. We also measured levels of glucose, insulin, leptin, glucagon, IGF-I, thyroid stimulating hormone (TSH) and the BAT-associated hormones fibroblast growth factor 21 (FGF-21) and irisin. The hormones insulin, leptin and IGF-I are capable of lowering serum glucose levels. Thyroid hormone receptors are present in BAT and higher levels of thyroid hormone are associated with higher levels of cold-activated BAT [22]. FGF-21 is associated with increased UCP-1 activity and produced by the liver, pancreas, WAT and BAT. Irisin is a protein produced when exercising muscles that activates UCP-1 and is capable of fat browning [23].

Materials and methods

Subjects

From an initial cohort of 82 subjects, this study included 30 healthy study subjects with a median age of 21 years (range, 19–22 years). Among these subjects, 10 had been born extremely preterm at <28 gestational weeks (GW), 10 were born full term (37–42 GW) but SGA and 10 were born full term with a normal birth weight (controls). These subjects, including controls, were part of an earlier study group [24]. The preterm individuals were selected from subjects with the lowest gestational age (GA), with an equal gender distribution. Of the 15 adults born preterm with lowest GA, five had open ductus arteriosus after birth and had metallic clips (contraindication for MRI) as a consequence of heart surgery performed as neonates and were thus excluded from participation. The other 10, five men and five women agreed to participate in the study. Controls were selected from those who had equivalent body mass index (BMI) values as the preterms. All 10 controls agreed to participate, as did all 10 SGA subjects (five men and five women) who had BMIs equivalent to the other groups. All subjects were of Caucasian origin. GA was determined by ultrasound during early pregnancy [25]. SGA was defined as having a birth weight of less than 2 standard deviations (SD) below the mean [26].

All participants were singletons born during 1990–1993, and none of them had chromosomal anomalies, congenital infections or life-threatening congenital anomalies. All the three groups had equivalent BMI values. Anthropometric data are presented in Table 1. Standard deviation scores (SDS) were based on the Swedish reference curve at birth [26] and adulthood (Table 1). All participants gave their written informed consent and the study was approved by the regional Ethical Committee.

Blood sampling

Blood sampling was performed in the morning after an overnight fast, and serum was stored at -70°C until analysis. Assays for irisin and FGF-21 were performed using enzyme-linked immunosorbent assay (ELISA) kits from BioVendor (RAG018R; Brno, Czech Republic) and R&D Systems, Inc. (DF2100; Minneapolis, MN, USA), respectively, following the manufacturers' instructions. The detection limit for irisin was 1 ng/mL, the intra- and inter-assay coefficients of variation (CV) were 4.8–8.2% and <10%, respectively. The detection limit for FGF-21 was 8.7 pg/mL, the intra- and inter-assay CVs were 3.9% and 5.2–10.9%, respectively. TSH was analyzed using the Access HYPERSensitive hTSH assay (detection limit 0.01 mE/L, the intra- and inter-assay CVs were 4.8% and 4.4%, respectively) and free thyroxine (T4) using the Access Free T4 assay (detection limit 3.2 pmol/L, the intra- and inter-assay CVs were 8.1% and 4.4%, respectively) both from Beckman Coulter, Inc. (Brea, CA, USA). Glucose was analyzed by photometry following routine laboratory protocols. Leptin and insulin were analyzed by radioimmunoassay using the HL-81K (Linco Research, Inc., St. Charles, MO, USA) and electrochemiluminescence immunoassays (Roche Diagnostics GmbH, Mannheim, Germany), respectively. The sensitivity of detection for leptin was 0.5 mg/L. The intra-assay CV for the leptin analysis was 5%. The inter-assay CV was 4.5%. The detection limit for insulin was 3 pmol/L. The intra- and inter-assay CVs for insulin were 6.7% and 7.5%, respectively. Glucagon was analyzed using the

Table 1: Anthropometric data.

	Preterm (n=10)	SGA (n=10)	Control (n=10)
Sex, women/men, n	5/5	5/5	5/5
Birth weight, g	905 (850–960)	2385 (2130–2640)	3475 (3200–3750)
Birth weight SDS	−0.40 (−2.13 to 0.92)	−3.28 (−4.18 to −2.18)	−0.34 (−1.94 to 0.68)
Gestational age, weeks	25.6 (25–26)	39.2 (37.9–40.5)	39.8 (39.1–40.5)
Age at follow-up, years	21.3 (20.8–21.8)	20.4 (19.6–21.2)	20.4 (19.6–21.2)
BMI, kg/m ²	22.3 (20.1–24.4)	22.4 (20.9–24.0)	21.7 (19.8–23.6)
Height, cm	171.5 (163–180)	169.5 (164–175)	173.9 (166–182)
Height SDS	−0.59 (−1.59 to 1.76)	−1.10 (−1.74 to 0.70)	−0.43 (−1.25 to 1.76)
Weight, kg	65.2 (56–74)	64.4 (59–70)	65.9 (58–74)
Weight SDS	0.28 (−2.78 to 1.16)	−0.13 (−1.75 to 1.04)	0.27 (−1.62 to 1.06)

Values are presented as mean (95% CI). BMI, body mass index; SDS, standard deviation score.

EURIA RIA kit (Euro Diagnostica, Malmo, Sweden). The detection limit was 3 pmol/L, and the intra- and inter-assay CVs were 8.1% and 8.3%, respectively. IGF-I was analyzed using commercial IGF-I assay (IDS-iSYS, Immunodiagnostic Systems, Tyne and Wear, United Kingdom). The detection limit was 9 µg/L and the intra- and inter-assay CVs were 2% and 4% at 163 µg/L, and 4% and 8% at 304 µg/L, respectively. The IGF-I SDS was calculated relative to a reference cohort.

MRI

MRI was performed between December and February (in northern Europe), without substantial differences in outside temperature among measurement dates. BAT is considered to be most active at this time of the year due to the low outside temperature [27]. Subjects were examined in the supine position with arms along the body at 21 °C in a 3T MRI scanner (Verio; Siemens Healthcare, Erlangen, Germany). The juxtaposition of soft tissue, lungs and air in the supraclavicular and cervical regions can create susceptibility effects that make imaging difficult. To compensate for this, we used an MRI technique that combines multiple scans to enable smaller echo spacing [28, 29] and an advanced fat-water separation method that uses graph cuts to estimate B_0 inhomogeneity and incorporates a multipeak fat spectral model [30].

The sequence was validated on fat-water phantoms with known fat fractions. We acquired and combined three offset acquisitions of

4 min and 55 s each from a dual-gradient echo sequence: echo time (TE_1)=1.88 ms and TE_2 =3.69 ms; TE_1 =2.29 ms and TE_2 =4.10 ms; and TE_1 =2.74 ms and TE_2 =4.55 ms. Coronal images of the supraclavicular/cervical region were obtained with a free-breathing three-dimensional dual-gradient echo sequence: flip angle=3°, repetition time=21 ms, 104 slices, field of view=377×377 mm, voxel size=2×2×2 mm, generalized autocalibrating partially parallel acquisition=2 and bandwidth=1530 Hz/px. Center frequency, radio frequency power and shims were fixed for all three acquisitions and monopolar gradients were used.

PDFF and $R2^*$ maps were analyzed using in-house developed software. Fat in both supraclavicular fossae and lower cervical regions was manually segmented in the PDFF maps (Figure 1) by an experienced radiologist in musculoskeletal imaging. Coronal and axial T2-weighted images were used for anatomical reference. Great care was taken to not include vessels, nerves or muscles in the region of interest (ROI) and to avoid partial volume artifacts in the periphery. The supraclavicular fossae are anatomically easily outlined by the trapezoid and the sternocleidomastoid muscles [9]. The most caudal part of the supraclavicular region was defined at the level of the superior part of the medial part of the clavicle. The most cranial position was in the lower cervical region, which depended on the extension of supraclavicular fat for each patient. ROIs were used to calculate PDFF, $R2^*$ and fat volume for each patient. For comparison, an ROI was placed in the dorsal subcutaneous tissue (WAT) of each patient's thorax to calculate PDFF and $R2^*$.

Figure 1: Representative coronal MRI PDFF maps of supraclavicular/cervical fat tissue from each group.

(A) Preterm (small volume), (B) SGA (large volume) and (C) control (moderate volume). Red lines represent the most caudal part of the supraclavicular region.

Statistics

Values for PDFF, R2* and supraclavicular/cervical fat volume are presented as mean and 95% confidence interval (CI). Irisin, leptin, insulin, glucagon and IGF-I values were not normally distributed; thus, they were log-transformed before analysis and are presented as geometric mean and 95% CI. Between-group comparisons were performed using analysis of variance (ANOVA). The Wilcoxon matched-pairs test was used to compare PDFF for supraclavicular/cervical and subcutaneous fat within the groups. A p-value of less than 0.05 was considered to indicate significance. The results were analyzed using StatSoft version 10 (Tulsa, OK, USA).

Results

Table 2 and Figure 2 show the measurements of supraclavicular/cervical fat subcutaneous adipose tissue, and

Table 3 shows the hormone levels. The three groups did not significantly differ with regard to supraclavicular/cervical PDFF, R2*, fat volume or hormone levels. In all the three groups, supraclavicular/cervical PDFF was significantly lower than subcutaneous adipose tissue PDFF ($p < 0.01$). Figure 3 shows PDFF histograms for each group. Between genders, no differences were found in supraclavicular/cervical PDFF % (mean [95% CI]; men 76% [74–77%] [$n = 15$] and women 77% [75–79%] [$n = 15$], [$p = 0.26$]).

Discussion

BAT measured by MRI showed no significant difference within or between the three investigated groups of young adults: extremely preterm, SGA and controls. Neither was there any significant difference in levels of the

Table 2: Measurements.

	Preterm (n=10)	SGA (n=10)	Control (n=10)	p-Value
PDFF supraclavicular/cervical fat, %	76 (74–79)	75 (73–77)	78 (75–80)	0.40
R2* supraclavicular/cervical fat	105 (98–111)	107 (101–114)	105 (99–112)	0.88
PDFF WAT, %	93 (92–95)	92 (90–94)	93 (91–95)	0.64
R2* WAT	71 (65–77)	74 (68–80)	68 (62–74)	0.62
Supraclavicular/cervical fat volume, mL	16 (8–24)	16 (8–24)	17 (9–25)	0.64

Values are presented as mean (95% CI). PDFF, proton density fat fraction; WAT, white adipose tissue.

Figure 2: Individual subcutaneous and supraclavicular fat fractions and supraclavicular fat volumes in the separate groups.

The left panel shows the mean PDFFs of subcutaneous white adipose tissue (WAT) and supraclavicular/cervical adipose tissue. Black-filled circles represent WAT and black-filled triangles represent supraclavicular/cervical adipose tissue in each individual in the indicated groups. The right panel shows the supraclavicular/cervical fat volume. The open circles represent the values of each individual in the indicated groups.

Table 3: Hormones.

	Preterm (n = 10)	SGA (n = 10)	Control (n = 10)	p-Value
Irisin, $\mu\text{g/mL}$	3.3 (3.0–3.7)	3.1 (2.8–3.4)	2.9 (2.6–3.2)	0.15
FGF-21, pg/mL	48 (24–99)	53 (26–108)	38 (18–77)	0.78
TSH, mE/L	2.2 (1.6–3.0)	1.8 (1.3–2.5)	2.0 (1.4–2.0)	0.70
Free T4, pmol/L	12 (11–13)	11 (10–12)	11 (10–12)	0.68
Glucose, mmol/L	4.8 (4.5–5.1)	4.8 (4.5–5.1)	4.7 (4.4–5.0)	0.83
Insulin, mmol/L	8.1 (5.8–11.4)	8.1 (5.3–12.2)	6.5 (4.3–10.0)	0.60
Leptin, $\mu\text{g/L}$	9.5 (5.3–17.3)	9.1 (3.3–25.3)	5.8 (2.8–11.8)	0.55
Glucagon, pmol/L	69 (63–75)	64 (59–70)	70 (64–76)	0.37
IGF-I, $\mu\text{g/L}$	284 (240–336)	246 (206–294)	241 (204–286)	0.33
IGF-I SDS	0.67 (–0.02 to 1.36)	–0.24 (–0.98 to 0.48)	–0.25 (–0.94 to 0.44)	0.11

Values are presented as mean (95% CI). FGF-21, fibroblast growth factor 21; TSH, thyroid stimulating hormone; T4, free thyroxine; IGF-I, insulin-like growth factor 1; SDS, standard deviation score.

Figure 3: PDFF (%) histograms for all the groups. (A) Preterm, (B) small for gestational age (SGA) and (C) controls. Supraclavicular/cervical brown adipose tissue (BAT) is shown in black color and white adipose tissue (WAT) in gray color.

BAT-associated hormones IGF-I, FGF-21 and irisin. These results support that BAT is present in young adults born extremely preterm or SGA.

BAT has been examined in several studies with MRI PDFF, including some post-mortem investigations, with a reported BAT PDFF range of 30–83%. The lowest PDFF has been reported in infants, who have the highest amount of BAT, while children and adolescents show a PDFF range of 65–73%, and in middle-aged the PDFF was 83% [19, 20, 31–33]. Our present results showed that the mean BAT PDFF in all the three investigated groups ranged from 75% to 77%, which is in concordance with an age-related decrease

in BAT, and with a low SD of 3%. This indicates that our applied MRI technique used in this study is a reliable method for PDFF assessment, even though this region can be difficult to image due to susceptibility effects. In a study on 11 elderly participants (mean age 80 years) with ^{18}F -FDG-negative PET-MRI, the fat fraction in supraclavicular tissue was 90%, which is in compliance with no residual BAT [21]. This finding is supported in a study where no ^{18}F -FDG PET BAT activation was seen after cold exposure in a 45 year old with a fat fraction in supraclavicular tissue of 87% [17].

The mean WAT PDFF in our study (range, 92–93%) was slightly higher than that reported in previous studies (range, 81–89%) [19, 20, 31], and closer to the expected value of 98% determined by MRI spectroscopy [34]. This can be attributed to our careful avoidance of partial volume effects during ROI placement, combined with a multi-peak triglyceride model [35]. Interestingly, only small amounts of voxels in the supraclavicular/cervical region had a PDFF of >90% (Figure 3), as seen in WAT. This could indicate that this region contained only sparse amounts of regular WAT. As expected, the mean $R2^*$ was higher in BAT than in WAT due to the high vascularization and iron-rich mitochondria of BAT [19, 36]. Although our study showed that BAT existed in young adults born SGA and extremely preterm, data from a different study suggest that there is a correlation between less BAT and cardiovascular disease in middle-aged patients [33].

A larger sample size might have found associations with the BAT-associated hormones IGF-I, FGF-21 and irisin. As there were no major differences in hormones between the groups, the hormonal findings are unlikely to explain the associations with BAT characteristics.

Our study has limitations. This is a pilot study and due to the small size of the study population, our conclusions must be viewed with caution and can only be considered

as preliminary data. However, even with our small sample size, our findings present substantial evidence contradicting the hypothesis that young adults born extremely preterm or SGA show disrupted BAT formation.

In conclusion, we demonstrate the presence of BAT in young adults born extremely preterm or SGA in northern Europe, thereby suggesting that an absence of BAT does not explain the increased risk for developing cardiovascular and metabolic disorders in these populations. However, with our small study cohort, our data cannot exclude that BAT may be involved in this scenario to a certain extent.

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