HHS Public Access

Author manuscript

Pediatr Obes. Author manuscript; available in PMC 2020 February 18.

Published in final edited form as:

Pediatr Obes. 2019 February ; 14(2): e12486. doi:10.1111/ijpo.12486.

Initial evidence for hypothalamic gliosis in children with obesity by quantitative T2 MRI and implications for BOLD response to glucose ingestion

Leticia E. Sewaybricker[1,2], **Ellen A. Schur**[2], **Susan J. Melhorn**[2], **Brunno M. Campos**[3], **Mary K. Askren**[4], **Guilherme A. S. Nogueira**[5], **Mariana P. Zambon**[1], **Maria Angela R. G. M. Antonio**[1], **Fernando Cendes**[3], **Licio A. Velloso**[5], **Gil Guerra-Junior**[1]

[1]Department of Pediatrics, School of Medical Sciences, University of Campinas, Campinas, Brazil

[2]Department of Medicine, University of Washington, Seattle, Washington, USA

[3]Neuroimaging Laboratory, Department of Neurology, University of Campinas, Campinas, Brazil

[4]Department of Radiology, University of Washington, Seattle, Washington, USA

[5]Laboratory of Cell Signaling, Department of Internal Medicine, University of Campinas, Campinas, Brazil

Abstract

Objective: In adults, hypothalamic gliosis has been documented using quantitative T2 neuroimaging whereas functional magnetic resonance imaging (fMRI) has shown a defective hypothalamic response to nutrients. No studies have yet evaluated these hypothalamic abnormalities in children with obesity.

Methods: Children with obesity and lean controls underwent quantitative MRI measuring T2 relaxation time, along with continuous hypothalamic fMRI acquisition to evaluate early response to glucose ingestion.

Results: Children with obesity (N=11) had longer T2 relaxation times, consistent with gliosis, in the mediobasal hypothalamus (MBH) compared to controls (N=9; p=0.004). Moreover, there was a highly significant group*region interaction (p=0.002), demonstrating that signs of gliosis were specific to MBH and not to reference regions. Longer T2 relaxation times correlated with measures of higher adiposity including visceral fat percentage (p=0.01). Mean glucose-induced hypothalamic BOLD signal change did not differ between groups (p=0.11). However, mean left MBH T2 relaxation time negatively correlated with glucose-induced hypothalamic signal change ($p<0.05$).

Conclusion: Imaging signs of hypothalamic gliosis were present in children with obesity and positively associated with more severe adiposity. Children with the strongest evidence for gliosis

Corresponding Author: Leticia E. Sewaybricker, MD, PhD, University of Washington, 750 Republican St., Box 358062, Seattle, WA 98109, USA, leticker@u.washington.edu, Phone: +1 206 543.5357.

Conflicts of Interest. The authors declared no conflict of interest.

showed the least activation after glucose ingestion. These initial findings suggest that the hypothalamus is both structurally and functionally affected in childhood obesity.

Keywords

gliosis; hypothalamus; magnetic resonance imaging; obesity; pediatric obesity

Introduction

Rodent studies implicate hypothalamic inflammation in the pathophysiology of obesity [1–6]. Dietary excess due to consumption of high-fat diet (HFD) triggers an inflammatory response in the mediobasal hypothalamus (MBH), even before weight gain begins[2] and affects the function [1,3] and number [2] of key neurons involved in energy balance, i.e. proopiomelanocortic (POMC) cells. In the short term, diet–induced hypothalamic inflammation leads to neuronal resistance to anorexigenic factors such as leptin [7] and insulin [8]. However, prolonged consumption of HFD can lead to potentially irreversible neuronal damage [2,9], raising concern that such findings underlie a disrupted control of energy homeostasis and the high recurrence rates of obesity [2,4,9].

Hypothalamic inflammation involves the activation and proliferation of glial cells, a process called gliosis. Gliosis is the characteristic central nervous system response to injury, and its detection in humans by structural magnetic resonance imaging (MRI) is a mainstay of clinical neuroradiology. However, clinical MRI studies use visual identification of increased T2 signal intensity (brightness) to detect gliosis[10,11]. But unlike computed tomography (CT) images, the brightness or “signal” has no absolute reference, so tissue signal cannot be directly compared from one subject or one scan to another. For research purposes, quantitative T2 MRI (T2MR) can be used to convert the brightness seen on T2-weighted MR images into T2 relaxation times, measured in milliseconds. Longer T2 relaxation times are consistent with gliosis, and validation studies have shown T2 relaxation time is positively correlated with staining intensity of astrocytes in the MBH of HFD-fed mice[4,] and in postmortem human MBH tissue [12]. Using T2MR, previous *in vivo* studies using this technique have shown that T2 signal is increased in the MBH of adult humans in association with obesity [2,12,13] and insulin resistance [12], which also suggests potential implications of hypothalamic gliosis for glucose regulation[12]. Correspondingly, distinct MRI approaches have been used to demonstrate abnormalities in obesity for both brain’s macrostructure (e.g. cortical thickness and regional volume)[14,15] and functionality[16–18]. For example, functional MRI (fMRI) studies in adults with obesity suggest a disrupted hypothalamic response to glucose ingestion characterized by an attenuated response after glucose intake[19,20]. It is unknown if children with obesity present the same response pattern to glucose ingestion. Thus, neuroimaging provides a minimally invasive approach to investigate hypothalamic function when obesity and/or hypothalamic gliosis are present.

Given the major epidemiological importance of childhood obesity, as a risk factor for adult obesity and comorbidities such as type 2 diabetes (T2D)[21], and the absence of data on hypothalamic gliosis in children, we employed structural (quantitative T2 imaging) and functional (BOLD response to glucose ingestion) neuroimaging techniques to evaluate the

presence of gliosis in the hypothalamus of children with obesity and its potential functional significance. We hypothesized that children with obesity would present signs of hypothalamic gliosis and a disrupted functional response to glucose ingestion.

Methods

Patients

Sixteen patients (7 females; 9–17 years old) with obesity (BMI >95th percentile CDC reference for age and sex) and no comorbidities were recruited from a convenience sample at the Pediatric Obesity Clinic at the University of Campinas Hospital. Fourteen healthy lean controls (7 females; 9–17 years old, BMI 5th–85th percentile) were recruited from the same hospital during routine preventive visits. Exclusion criteria for both groups were: T2D according to the American Diabetes Association criteria, neurologic or psychiatric disorders, kidney or liver diseases, inflammatory or infectious disease, body weight > 120kg (MRI limit) and permanent metallic implant. Seven subjects failed MRI acquisition (5 felt uncomfortable/claustrophobic during the scan and 2 had to be dismissed due to artifact caused by orthodontia). Movement artifact caused 3 structural and 2 functional scans from lean controls to be excluded from the respective analyses. Therefore, the final sample included 23 subjects from which 18 subjects (11 patients with obesity, 7 controls) had complete MRI outcomes from all acquisitions. The study was approved by the University of Campinas Ethics Committee. Participants and their legal guardians provided written informed assent and consent, respectively.

Procedures

Morning blood collection (8AM) and anthropometric and total body composition measurements (iDXA, enCore software, version 13.60, GE Healthcare Lunar, WI, USA) were performed after a 12-hour fast. A single pediatrician (LES) performed all interviews and physical evaluations. Participants were instructed not to change their daily routine between study visits. Puberty was defined by Tanner stage[22] (1–2 pre-puberty, ≥3 puberty). BMI Z-scores were determined using CDC reference.

Plasma concentrations of leptin, adiponectin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and −10 (IL-10) were determined by ELISA (R&D Systems, MN, USA) as well as total insulin (Millipore, MO, USA).

One week later, participants completed the MRI session, also preceded by a 12-hour fast, with a scan start time of 7–10AM. All scans were acquired on a 3-Tesla Phillips Achieva scanner (Phillips Medical Systems, Best, The Netherlands) using an 8-channel head radiofrequency coil according to the following protocol: i) T1-weighted image (WI) with isotropic voxels of 1mm, acquired in the sagittal plane, 180 slices, 1mm thick, no gap, flip angle = 8°, TR = 7.0ms, TE = 3.2ms, FOV = 240 × 240mm^2; ii) quantitative multi-slice/multi-echo T2-WI with 16 echoes, TE = 6.27ms, and TR = 2000ms, 11 slices were obtained from the optic chiasm through the mammillary bodies, 2mm thick and interslice gap = 0.071mm, voxel sizes of 1 × 1 × 2mm^3, and FOV = 256 × 240mm^2; iii) echo planar image (EPI) T2*-WI with 40 axial slices without intermission, TR = 2000ms, TE = 30ms, with

isotropic voxels of 3mm³, and FOV = 240 × 240mm^2. For each participant's EPI data, 1050 volumes were continuously acquired over a total of 35 minutes. During image acquisitions, subjects remained in a supine position with soft tape and foam pads used for head immobilization and were instructed to keep eyes closed. A 1.2m plastic tube was positioned in the oral cavity before imaging began to allow for the intake of dextrose glucose solution (dose of 1.75g/kg [up to 75g] diluted in 200mL of water) as previously described[18]. Ten minutes after the fMRI scan started, a physical signal was given, by a gentle touch in the subject's left leg, to initiate the glucose solution intake (time was monitored by the researcher [LES]).

fMRI data were preprocessed with Statistical Parametric Mapping software version 12 (Wellcome Trust Centre for Neuroimaging, London, UK). The images were initially realigned and six rigid-body parameters (three translational and three rotational) for motion correction were applied. Slice timing correction was performed with slice 20 used as a reference. To account for possible bias introduced by the degree of the reslicing and normalization transformations due to displacement, we performed a quantitative analysis of the realignment parameters estimating the Framewise Displacement (FD), maximum translation, maximum rotation as well as the cumulative displacement. FD threshold was defined as 0.5 mm. Functional images and high resolution T1 were co-registered for each participant and then normalized for the MNI-152 standard space (Montreal Neurological Institute template). Finally, a full width at half maximum (FWHM) of 6mm^3 smoothing was applied.

Gliosis evaluation by quantitative T2MR

Twenty participants had successful T2MR acquisitions (11 patients, 9 controls). T2 relaxation time was determined from the quantitative structural sequence as previously described[12].

The coronal slice immediately posterior to the optic chiasm was selected and regions of interest (ROI) including the MBH and two reference regions (amygdala and putamen) were positioned bilaterally for each participant on high resolution images then transferred to the parametric map, which was calculated from the signal decay curve of 16 echoes on a pixel-by-pixel basis (Figure 1A). T2 relaxation time (mean value and standard deviation) was obtained for each ROI (OsiriX Imaging Software, version 5.6). For correlational analyses, the mean bilateral value was calculated from left and right sides for each ROI and all participants were included (patients and controls).

Hypothalamic BOLD response to glucose

Twenty-one participants had successful fMRI acquisitions (11 patients, 10 controls). To assess the functional response to a glucose load, Blood-Oxygen Level Dependent (BOLD) signal from the hypothalamus was acquired from a manually drawn mask (112mm^3 for each side) created using the anatomical descriptions by Baroncini et al[23]. Pre-glucose signal intensity was obtained during a 10-minute period (309 volumes) before glucose ingestion. BOLD signal was excluded during the 3 minutes of glucose ingestion due to motion related to swallowing. Three additional minutes were excluded from analysis due to continued

artifact from swallowing movements. For analytic purposes, the signal intensity of a 10-minute post-glucose intake period (from 6–16 minutes, consisting of 309 volumes) was selected (Figure 2A). This time frame was chosen based in previous work described by Matsuda et al[19] showing a predicted decrease in BOLD signal in humans occurring 8–12 min after glucose intake initiation and data from van de Sande-Lee et al.[20] showing abnormal BOLD signal 10–20 min after glucose intake initiation in obese adults. Due to excessive motion, data obtained 20–25 minutes post-glucose intake was unanalyzable. Each time series (pre- and post-glucose BOLD signal) was normalized to the BOLD signal of its first volume respectively to allow comparison among participants by converting the arbitrary signal baseline from BOLD imaging to a percentile variation from baseline. For each participant, we calculated a mean pre-glucose hypothalamic BOLD signal from their normalized 309 volumes pre-glucose scan. This value represented the baseline pre-glucose BOLD activation. To capture minute by minute variability post-glucose ingestion, the 309 volumes of the post-glucose hypothalamic BOLD signal were divided and averaged over each of the 10-minute post-glucose BOLD activation acquisition periods. Glucose-induced hypothalamic BOLD signal change was determined by first subtracting each subject's mean pre-glucose baseline value from each volume of their 10-minute post-glucose acquisition period, then, these values were averaged to represent each subject's glucose-induced hypothalamic BOLD signal change (post minus pre-glucose). For correlational analyses, individual mean glucose-induced hypothalamic signal change was used to associate anthropometric measures and T2 relaxation times with changes in activation in response to glucose ingestion.

Statistical analysis

All data were analyzed using SPSS version 22.0 (SPSS Statistics Inc., NY, USA). For group comparisons, Student's t test and Mann-Whitney U test were used or Fisheŕs exact test (FET) for categorical variables. Pearson's were applied for correlation analyses and Spearman's rank correlation coefficients on non-normally distributed and unadjusted variables. Linear regressions were adjusted for age, sex, and puberty. When using Z-scores, linear regressions were adjusted for puberty. For pre- and post-glucose BOLD signal curves, a linear mixed model for repeated measures was applied to check the effect of glucose ingestion, over time, on each group. The mean pre-glucose BOLD signal baseline was used as a covariate in the model, as well as the average per minute (10 time points) for the post-glucose hypothalamic BOLD signal. For T2MR data, linear mixed models were used to test group differences across the 3 brain ROIs and group*region interaction. Both linear mixed models were completed with STATA (StataCorp LLC. Release 15. TX, USA) using adjustment for small samples based on Kenward and Roger's method[24]. Statistical significance was determined at $p<0.05$. We also present Bonferroni corrected P-values adjusted (p_{adj}) for 5 measures of body adiposity.

Results

General characteristics

Groups were well-matched for age, height, sex (FET p=1.00, females: 46% patients; 50% controls), and puberty (FET p=0.21, pre-puberty: 55% patients, 25% controls). Lean mass

was similar between groups whereas total fat mass, visceral fat mass, and android (but not gynoid) fat percentages were greater in children with obesity. Furthermore, children with obesity had higher plasma concentrations of leptin, TNF-α, and IL-6, whereas plasma adiponectin was higher in controls. Despite higher adiposity in children with obesity, there was no difference in fasting insulin concentrations between groups (Table 1).

Structural abnormalities in the hypothalamus of children with obesity

Linear mixed model analyses of mean bilateral T2 relaxation time demonstrated a trend for an effect of group ($F_{1,21}$=3.79, p=0.065), a main effect for region ($F_{2,37}$=552.58, p<0.001), and a highly significant group*region interaction ($F_{2,37}$=7.23, p=0.002), independent of age. Due to the presence of the interaction, formal *post hoc* testing was performed to test group differences within each region. Analyses revealed significantly longer mean bilateral T2 relaxation times in children with obesity compared to controls in the MBH, but not in the reference regions (Table 1, Figure 1B). Given previous lateralized findings[4,13] we examined each side separately. Children with obesity had longer mean T2 relaxation times for the left and right MBH compared to controls and MBH T2 relaxation times were longer on the right than left MBH for both groups (Table 1). No effect of side was found for putamen and amygdala (data not shown). Prior studies document that T2 relaxation times decrease in normal brain tissue during childhood and adolescence, an effect that is considered to be related to physiologic changes during brain maturation[25]. Accordingly, mean bilateral T2 relaxation time was negatively correlated with age for all participants within both reference regions (amygdala r=−0.76, p<0.001; putamen r=−0.49, p=0.03) but not for MBH (r=−0.31, p=0.19). But, when groups were analyzed separately, controls did demonstrate the expected negative association in the MBH, but no correlation was seen for children with obesity (Figure 1C). Linear regression analyses were used to test for an interaction, and a strong trend (t=2.09, p=0.053) suggested that the relationship between age and MBH T2 relaxation time differed when obesity was present. No effect of sex was seen for any ROI (data not shown).

In all participants (controls and patients), positive associations were found between mean bilateral MBH T2 relaxation time and BMI Z-score (rho:0.55, p=0.01, p_{adj}=0.06, Figure 1D), weight Z-score (Figure S1A), body fat percentage (r=0.53, p=0.04, p_{adj}=0.22, Figure 1E), visceral fat mass (rho=0.56, p=0.01, p_{adj}=0.06, Figure 1F) and android fat percentages (r=0.68, p=0.009, p_{adj}=0.045, Figure 1G). No correlation was found between T2 relaxation time from reference regions and adiposity measures (amygdala: BMI Z-score: rho=−0.07, p=0.77; Body fat %: r=0.12, p=0.60; Visceral fat %: rho=0.03, p=0.92 and Android fat %: r=−0.08, p=0.74; putamen: BMI Z-score: rho=0.01, p=0.97; Body fat %: r=−0.11, p=0.65; Visceral fat %: rho=−0.11, p=0.64 and Android fat %: r=−0.21, p=0.37). In addition, longer MBH T2 relaxation time was associated with increased plasma leptin concentrations (Figure S1B), but not fasting insulin (Figure S1C). Trends suggested potential relationship between mean bilateral MBH T2 relaxation time and inflammatory markers TNF-α and IL-6 (TNF-α: r=0.40, p=0.09; IL-6: rho=0.41, p=0.08) but not IL-10 (rho=0.27, p=0.26).

Relationships of obesity with hypothalamic BOLD response to glucose ingestion

No difference in mean BOLD signal was seen between groups during the 10-minute baseline period before glucose intake (patients 0.98±0.01; controls 0.97±0.02, p=0.34 [values represent the average of dotted lines in Figure 2B]). Also, mean Post-glucose BOLD signal did not differ between patients and controls (1.00±0.04 vs. 1.01±0.07 respectively, p=0.68, solid lines in Figure 2B).

Interestingly, both groups demonstrated visual increases in hypothalamic BOLD signal after glucose ingestion, especially at later time points (solid lines in Figure 2B). Therefore, to explore any effect of time in the BOLD signal after glucose intake, a linear mixed model with repeated measures was used and showed no significant effect for time post-glucose ($F_{9,171}$=0.62, p=0.78), group ($F_{1,18}$=1.00, p=0.33) or time*group interaction ($F_{9,171}$=0.80, p=0.61, Figure 2C). Although sample size was small for adjusted analyses, results did not differ when age was included as a covariate (data not shown).

The mean glucose-induced hypothalamic BOLD signal change did not differ between groups (patients 0.02±0.04; controls 0.05±0.05, p=0.11), neither did it correlate with BMI Z-score (rho=−0.12, p=0.96) or visceral fat percentage (rho=−0.26, p=0.25). Among all participants, there was a trend between age and Pre- (r=0.41, p=0.06) and Post-glucose BOLD signal (r=0.38, p=0.09). However, no correlation was seen between mean glucose-induced BOLD signal change and age (r=0.14, p=0.56).

Relationships of signs of gliosis and BOLD signal change after glucose intake within the hypothalamus

In order to test whether structural abnormalities were related to hypothalamic function after glucose ingestion, we compared MBH T2 relaxation time to the change in hypothalamic BOLD signal across all participants. T2 relaxation time in the left MBH was negatively correlated to the BOLD hypothalamic response to glucose, represented by the mean glucose-induced signal change (rho=−0.51; p=0.03; Figure 2D). The direction of correlation for the right MBH was also negative, but the relationship was attenuated (r=−0.17; p=0.50). There were no relationships between the mean glucose-induced BOLD signal change and T2 relaxation time in reference regions (putamen: left r=0.19, p=0.45; right r=0.12, p=0.65; amygdala: left r=−0.04, p=0.87; right r=0.12, p=0.63).

Importantly, the relationship between glucose-induced signal change and MBH gliosis appears to be a result of a stronger decrease in the post-glucose BOLD signal from baseline as both Pre- and Post-glucose BOLD signal were unrelated to the MBH T2 relaxation time (Pre: left β=0.20, p=0.41, right β=0.23, p=0.35; Post: left β=−0.31, p=0.20, right β=0.01, p=0.98, adjusted for age). This suggests that children with signs of left MBH gliosis present lower BOLD signal after glucose ingestion (Figure 2D).

Discussion

Using quantitative MRI, we found evidence for the presence of gliosis in the MBH of children with obesity. Specifically, longer T2 relaxation times were found in the MBH of patients with obesity compared to lean controls, a difference that was not observed in

reference brain areas (amygdala and putamen). In functional analyses performed before and after an oral glucose load, mean glucose-induced hypothalamic signal change did not differ when comparing children with obesity to controls. While hypothalamic functional neuronal response did not differ based on obesity alone, it did differ based on the presence of gliosis, such that less hypothalamic activation after glucose administration was associated with a greater degree of left MBH gliosis by MRI. In sum, the findings in this small study provide initial evidence that MBH gliosis is present in childhood obesity and has potential implications for hypothalamic function during glucose processing.

The findings corroborate a growing rodent literature[26] and human studies in adults demonstrating that MBH gliosis is present in obesity *in vivo*[2,12,13] and postmortem[27]. We expand upon these results to show that MBH gliosis may already be present in children with obesity, independent of age. Moreover, the association of gliosis with adiposity was also suggested for visceral fat deposition[28]. Thus, hypothalamic gliosis may be linked to fat deposition with negative metabolic consequences. As such, the findings are informative regarding previous results in humans showing greater insulin resistance among adults with gliosis, independent of body fat mass [12]. In addition, T2 values normally decline with age[25], a relationship that was present in reference areas in putamen and amygdala, but absent in the MBH of children with obesity. Instead, MBH T2 relaxation time was positively correlated with adiposity, lending further support to the argument that normal tissue composition is disrupted within this region in association with obesity.

Preclinical evidence also implicates inflammatory processes in the MBH with disruption in glucose metabolism [8]. However, findings in adult humans to date are conflicting, i.e., one study showed greater insulin resistance when radiologic evidence for hypothalamic gliosis was present [12] whereas a second study found no relationship [13]. In the current pediatric study, we did not detect a correlation between MBH T2 relaxation time and fasting insulin concentrations. This may have been due to the low levels of fasting insulin among the participants with obesity in this small study or the lack of a more precise method of estimating insulin sensitivity, such as a hyperinsulinemic clamp procedure. An alternative interpretation is that MBH gliosis directly promotes insulin resistance, but either the duration or severity of the MBH gliosis was insufficient in young children to produce the relationship to insulin resistance observed in adults [12]. Nonetheless, MBH gliosis might be an early finding in children that predisposes them to future insulin resistance indirectly via an effect favoring visceral fat deposition. In rodent models of DIO, inflammatory responses from glial cells, including astrocytes and microglia, have been shown to be necessary for the hyperphagia and weight gain seen during high fat feeding [5,6], but discerning if MBH gliosis is a marker or pathogenic component of obesity and insulin resistance in humans will require additional, longitudinal data, as the current cross sectional design limits such interpretations. In addition, dietary factors related to MBH gliosis in humans have yet to be defined and are important area for future research. Studies with rodent models have commonly implicated the HFD [1,2,5], but sucrose ingestion[29] and neonatal overnutrition[30] could also contribute to the inflammatory process and altered glucose metabolism.

In previous functional studies, the hypothalamus of adults with obesity responded anomalously to oral glucose, presenting an attenuated response by fMRI [19] when compared

to the expected signal decrease shown by healthy controls [18]. In the current small study, neither group showed significant modification in hypothalamic BOLD signal after glucose ingestion. This is consistent with a study including lean adolescents (N=14) that demonstrated no change in hypothalamic perfusion after glucose ingestion[31]; however the same study reported increased perfusion in adolescents with obesity (N=24)[31] In agreement with the latter, an increase in hypothalamic BOLD signal after glucose ingestion has also been described in a study with adults with obesity and lean subjects (total N=21)[20] and two studies involving only normal-weight adults [18,32] (N=18 and 7, respectively). Such discrepancies in prior findings of hypothalamic response to glucose ingestion may be due to distinct fMRI methodologies to evaluate the hypothalamic function (BOLD, cerebral blood perfusion, temporal cluster analyses); variability due to the small samples in these and the current study, and to the fact that the glucose stimulus triggers a complex peripheral and CNS response orchestrated by the hypothalamus[33]. In addition, body composition or even age-related differences may have influenced the divergent results. However, the current study suggests that prior discrepant findings could also be because hypothalamic gliosis, when present in obesity, might be a more critical factor than adiposity itself in disrupting central response to glucose ingestion.

Given the central role of MBH neuron populations in glucose regulation, hypothalamic functional impairment is a plausible consequence of the presence of gliosis. Such functional impairments (i.e., disrupted insulin signaling and glucose intolerance) have been linked to the presence of MBH gliosis in experimental models of diet-induced obesity[1,8]. However, in adult humans with obesity, there was no evidence of altered neuronal content in the MBH by MR spectroscopy despite T2-hyperintensities in the hypothalamus[13]. Thus, it remains uncertain, in humans, whether neuronal damage or the proliferation and activation of the glial cells underlie the observed functional differences. In our study, we found evidence for functional changes when MBH gliosis was present. Specifically, T2 relaxation time in the left MBH was negatively correlated with mean glucose-induced hypothalamic signal change. No correlations were found in reference regions, providing evidence that the presence of MBH gliosis, specifically, is related to impaired glucose processing in the hypothalamus. Given our small population size in each group, we were not able to reliably stratify this analysis by children with obesity and controls, and this limitation also increases the possibility that the magnitude of relationships in MBH and control regions could be overestimated or findings due to chance alone. Larger samples should assess for effect modification in this relationship. The lateralization of the finding to the left MBH gliosis is consistent with evidence that hypothalamic white matter connections are lateralized in humans [34] and that the parasympathetic output (contributing to glucose regulation) is predominant in the left side of the brain [35]. Previous studies have also demonstrated stronger associations of obesity with gliosis detected in the left MBH [2,13]. In this small sample, right sided T2 relaxation time were higher than left, although left-sided values were still elevated (compared to our control participants). The fact that relationships were seen between BOLD glucose response and MBH gliosis on the left side suggests both that the degree of alterations in T2 signal observed have potential functional consequences and that response to nutrients may be a lateralized function. Lateralization of hypothalamic function on feeding pathways has been suggested [36], but awaits further investigation in children.

Neuroimaging studies focused on the hypothalamus are technically challenging, since this is an anatomically small region, next to the sinuses and the 3rd ventricle, which may result in image artifacts [16]. Although it is not possible to distinguish between distinct hypothalamic nuclei due to limited MRI spatial resolution, gliosis in other hypothalamic areas (e.g., ventromedial or dorsomedial nuclei) could also be related to hypothalamic function after glucose ingestion. A further limitation in our study was to not have monitored glucose levels.

The ability to perform *in vivo* imaging in young children is a strength of the current study. Although not observed in this study, sex differences or puberty could influence relationships of gliosis to our outcomes[37] and studies with larger sample sizes are needed for stratified analyses to assess for sex-specific or developmental differences. Furthermore, T2MR is an indirect measure of tissue characteristics and cannot parse the cellular characteristics of the MBH. Animal models will likely be required to define the complex interplays of aging, brain maturation, and hypothalamic inflammation in childhood obesity pathogenesis. It is possible that the abnormal results from T2MR might also indicate other structural changes, such as edema and/or areas of sclerosis[12] which would nonetheless be abnormal findings in pediatric obesity. However, previous histopathological analyses in brain tissue demonstrate correlations between measures of high T2 signal and the cellular process of reactive gliosis [4,11,12] which bolster our interpretation that the current imaging findings of longer MBH T2 relaxation times, independent of age, are consistent with hypothalamic gliosis in children with obesity. Further studies correlating different imaging techniques (e.g., diffusion imaging for microstructure) and their association with histological findings should help to strengthen neuroimaging methods in the study of human obesity.

In conclusion, this is the first study to show structural abnormalities in the hypothalamus of a pediatric population with obesity and associate these abnormalities with alterations in hypothalamic functional response to glucose. Our data suggest that the human hypothalamus is affected early during the development and progression of obesity, which may play an important role in the high risk of obesity and comorbidities in adults who had obesity as children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements.

Support for the study was provided by the Sao Paulo Research Foundation (FAPESP) grant 2009/50809-5, Conselho Nacional de Desenvolvimento Cientifico e Tecnológico, and grants from the Trust in Science Initiative from Glaxo-SmithKline, UK. Additional support was provided by National Institute of Health grants R01DK089036 and R01DK098466 and the University of Washington Royalty Research Fund and Nutrition and Obesity Research Center (P30DK035816). The Laboratory of Cell Signaling belongs to the Obesity and Comorbidities Research Center and the National Institute of Science and Technology – Diabetes and Obesity. LES, LAV and GGJ designed and conceived the study. LES, BMC and GASN performed the experiment. All authors contributed for the analysis and interpretation of the data. LES, EAS, LAV and GGJ wrote the manuscript. All authors reviewed and edited the manuscript. We thank E. Roman and G. Ferraz from the University of Campinas for technical assistance. We also thank S. Barbosa and R. Mendes for assistance on DXA measurements.

References

1. De Souza CT, Araujo EP, Bordin S, et al. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology. 2005;146(10):4192–4199. doi:10.1210/en.2004-1520 [PubMed: 16002529]
2. Thaler JP, Yi C, Schur EA, et al. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest. 2012;122(1):153–162. doi:10.1172/JCI59660 [PubMed: 22201683]
3. Zhang X, Zhang G, Zhang H, Karin M, Bai H, Cai D. Hypothalamic IKKβ/NF-κB and ER Stress Link Overnutrition to Energy Imbalance and Obesity. Cell. 2008;135(1):61–73. doi:10.1016/j.cell.2008.07.043 [PubMed: 18854155]
4. Lee D, Thaler JP, Berkseth KE, Melhorn SJ, Schwartz MW, Schur E a. Longer T(2) relaxation time is a marker of hypothalamic gliosis in mice with diet-induced obesity. Am J Physiol Endocrinol Metab. 2013;304(11):E1245–50. doi:10.1152/ajpendo.00020.2013 [PubMed: 23548614]
5. Valdearcos M, Douglass JD, Robblee MM, et al. Microglial Inflammatory Signaling Orchestrates the Hypothalamic Immune Response to Dietary Excess and Mediates Obesity Susceptibility. Cell Metab. 2017;26(1):185–197.e3. doi:10.1016/j.cmet.2017.05.015 [PubMed: 28683286]
6. Douglass JD, Dorfman MD, Fasnacht R, Shaffer LD, Thaler JP. Astrocyte IKKβ/NF-κB signaling is required for diet-induced obesity and hypothalamic inflammation. Mol Metab. 2017;6(4):366–373. doi:10.1016/j.molmet.2017.01.010 [PubMed: 28377875]
7. Banks WA, Coon AB, Robinson SM, et al. Triglycerides induce leptin resistance at the blood brain barrier. Diabetes. 2004;53(8):1253–1260. doi:10.2337/diabetes.53.5.1253 [PubMed: 15111494]
8. Posey KA, Clegg DJ, Printz RL, et al. Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. Am J Physiol Endocrinol Metab. 2009;296(5):E1003–12. doi:10.1152/ajpendo.90377.2008 [PubMed: 19116375]
9. Moraes JC, Coope A, Morari J, et al. High-fat diet induces apoptosis of hypothalamic neurons. PLoS One. 2009;4(4):e5045. doi:10.1371/journal.pone.0005045 [PubMed: 19340313]
10. Marshall VG, Bradley WG, Marshall CE, Bhoopat T, Rhodes RH. Deep white matter infarction: correlation of MR imaging and histopathologic findings. Radiology. 1988;167(2):517–522. doi:10.1148/radiology.167.2.3357964 [PubMed: 3357964]
11. Braffman BH, Zimmerman RA, Trojanowski JQ, Gonatas NK, Hickey WF, Schlaepfer WW. Brain MR: pathologic correlation with gross and histopathology. 2. Hyperintense white-matter foci in the elderly. AJR Am J Roentgenol. 1988;151(3):559–566. doi:10.2214/ajr.151.3.559 [PubMed: 3261518]
12. Schur EA, Melhorn SJ, Oh S-K, et al. Radiologic evidence that hypothalamic gliosis is associated with obesity and insulin resistance in humans. Obesity (Silver Spring). 2015;23(11):2142–2148. doi:10.1002/oby.21248 [PubMed: 26530930]
13. Kreutzer C, Peters S, Schulte DM, et al. Hypothalamic Inflammation in Human Obesity Is Mediated by Environmental and Genetic Factors. Diabetes. 2017;66(9):2407–2415. doi:10.2337/db17-0067 [PubMed: 28576837]
14. Saute RL, Soder RB, Alves Filho JO, Baldisserotto M, Franco AR. Increased brain cortical thickness associated with visceral fat in adolescents. Pediatr Obes. 2018;13(1):74–77. doi:10.1111/ijpo.12190 [PubMed: 27788560]
15. de Groot CJ, van den Akker ELT, Rings EHHM, Delemarre-van de Waal HA, van der Grond J. Brain structure, executive function and appetitive traits in adolescent obesity. Pediatr Obes. 2017;12(4):e33–e36. doi:10.1111/ijpo.12149 [PubMed: 27241878]
16. Salem V, Dhillo WS. IMAGING IN ENDOCRINOLOGY: The use of functional MRI to study the endocrinology of appetite. Eur J Endocrinol. 2015;173(2):R59–68. doi:10.1530/EJE-14-0716 [PubMed: 25855629]
17. Schur EA, Kleinhans NM, Goldberg J, Buchwald D, Schwartz MW, Maravilla K. Activation in brain energy regulation and reward centers by food cues varies with choice of visual stimulus. Int J Obes (Lond). 2009;33(6):653–661. doi:10.1038/ijo.2009.56 [PubMed: 19365394]
18. Liu Y, Gao JH, Liu HL, Fox PT. The temporal response of the brain after eating revealed by functional MRI. Nature. 2000;405(6790):1058–1062. doi:10.1038/35016590 [PubMed: 10890447]

19. Matsuda M, Liu Y, Mahankali S, et al. Altered hypothalamic function in response to glucose ingestion in obese humans. Diabetes. 1999;48(9):1801–1806. http://www.ncbi.nlm.nih.gov/pubmed/10480611. [PubMed: 10480611]
20. van de Sande-Lee S, Pereira FRS, Cintra DE, et al. Partial reversibility of hypothalamic dysfunction and changes in brain activity after body mass reduction in obese subjects. Diabetes. 2011;60(6):1699–1704. doi:10.2337/db10-1614 [PubMed: 21515852]
21. Daniels SR. Complications of obesity in children and adolescents. Int J Obes (Lond). 2009;33(S1):S60–5. doi:10.1038/ijo.2009.20
22. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child. 1976;51(3):170–179. doi:10.1136/adc.51.3.170 [PubMed: 952550]
23. Baroncini M, Jissendi P, Balland E, et al. MRI atlas of the human hypothalamus. Neuroimage. 2012;59(1):168–180. doi:10.1016/j.neuroimage.2011.07.013 [PubMed: 21777680]
24. Kenward MG, Roger JH. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics. 1997;53(3):983–997. http://www.ncbi.nlm.nih.gov/pubmed/9333350. [PubMed: 9333350]
25. Hasan KM, Walimuni IS, Abid H, et al. Multimodal Quantitative Magnetic Resonance Imaging of Thalamic Development and Aging across the Human Lifespan: Implications to Neurodegeneration in Multiple Sclerosis. J Neurosci. 2011;31(46):16826–16832. doi:10.1523/JNEUROSCI.4184-11.2011 [PubMed: 22090508]
26. Valdearcos M, Xu AW, Koliwad SK. Hypothalamic Inflammation in the Control of Metabolic Function. Annu Rev Physiol. 2015;77(1):131–160. doi:10.1146/annurev-physiol-021014-071656 [PubMed: 25668019]
27. Baufeld C, Osterloh A, Prokop S, Miller KR, Heppner FL. High-fat diet-induced brain region-specific phenotypic spectrum of CNS resident microglia. Acta Neuropathol. 2016;132(3):361–375. doi:10.1007/s00401-016-1595-4 [PubMed: 27393312]
28. Berkseth K, Rubinow K, Melhorn S, et al. Hypothalamic gliosis by MRI and visceral adiposity negatively correlate with plasma testosterone concentrations in healthy men. Obes (Silver Spring). 2018.
29. Fuente-Martín E, García-Cáceres C, Díaz F, et al. Hypothalamic inflammation without astrogliosis in response to high sucrose intake is modulated by neonatal nutrition in male rats. Endocrinology. 2013;154(7):2318–2330. doi:10.1210/en.2012-2196 [PubMed: 23671260]
30. Tapia-González S, García-Segura LM, Tena-Sempere M, et al. Activation of Microglia in Specific Hypothalamic Nuclei and the Cerebellum of Adult Rats Exposed to Neonatal Overnutrition. J Neuroendocrinol. 2011;23(4):365–370. doi:10.1111/j.1365-2826.2011.02113.x [PubMed: 21314736]
31. Jastreboff AM, Sinha R, Arora J, et al. Altered Brain Response to Drinking Glucose and Fructose in Obese Adolescents. Diabetes. 2016;65(7):1929–1939. doi:10.2337/db15-1216 [PubMed: 27207544]
32. Flanagan DE, Fulford J, Krishnan B, Benattayallah a., Watt a., Summers IR. Functional MRI of the hypothalamic response to an oral glucose load. Diabetologia. 2012;55(7):2080–2082. doi:10.1007/s00125-012-2559-4 [PubMed: 22538360]
33. Watts AG, Donovan CM. Sweet talk in the brain: glucosensing, neural networks, and hypoglycemic counterregulation. Front Neuroendocrinol. 2010;31(1):32–43. doi:10.1016/j.yfrne.2009.10.006 [PubMed: 19836412]
34. Lemaire J-JJ, Frew AJ, McArthur D, et al. White matter connectivity of human hypothalamus. Brain Res. 2011;1371:43–64. doi:10.1016/j.brainres.2010.11.072 [PubMed: 21122799]
35. Wittling W, Block A, Genzel S, Schweiger E. Hemisphere asymmetry in parasympathetic control of the heart. Neuropsychologia. 1998;36(5):461–468. http://www.ncbi.nlm.nih.gov/pubmed/9699952. [PubMed: 9699952]
36. Wright H, Li X, Fallon NB, et al. Differential effects of hunger and satiety on insular cortex and hypothalamic functional connectivity. Eur J Neurosci. 2016;43(9):1181–1189. doi:10.1111/ejn.13182 [PubMed: 26790868]

37. Carnell S, Gibson C, Benson L, Ochner CN, Geliebter A. Neuroimaging and obesity: current knowledge and future directions. Obes Rev. 2012;13(1):43–56. doi:10.1111/j.1467-789X.2011.00927.x [PubMed: 21902800]

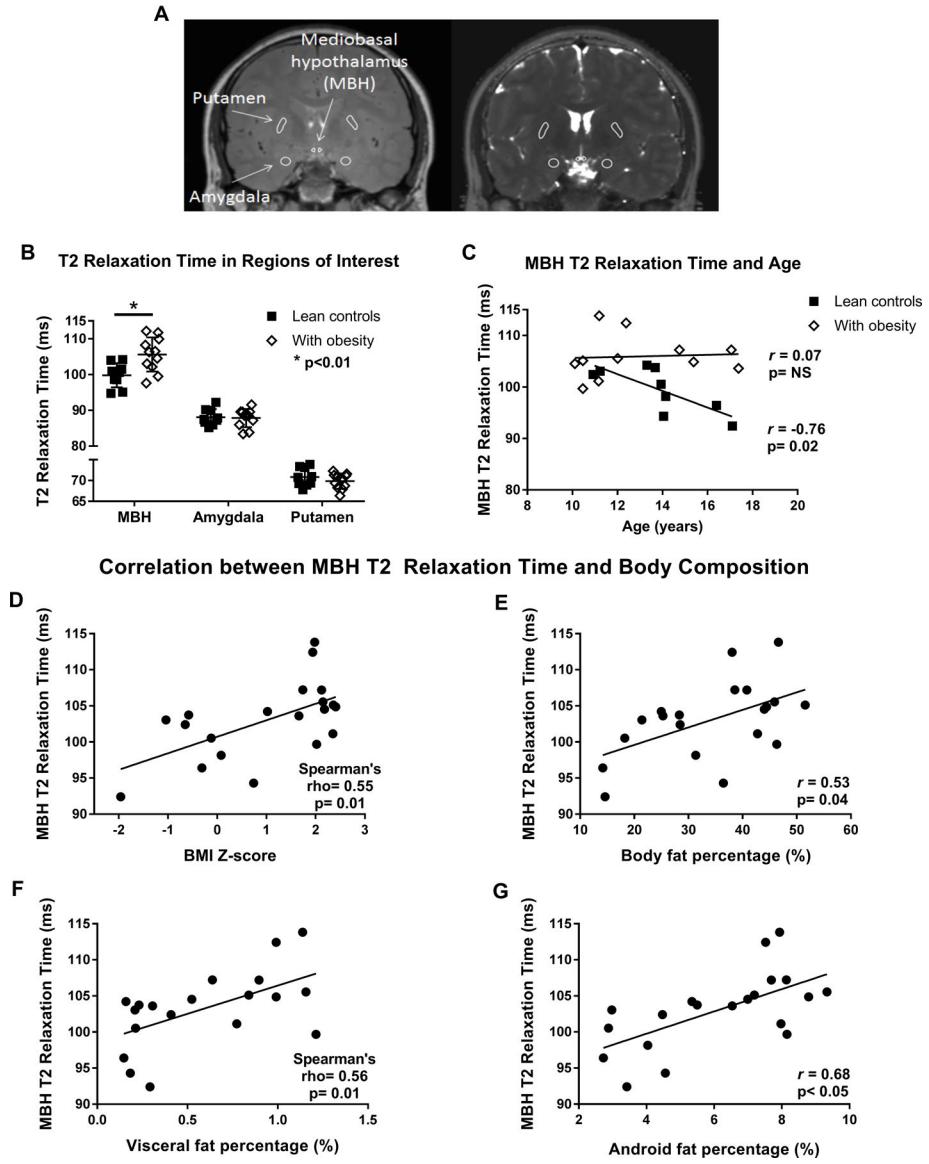

Figure 1. Measurement of T2 relaxation time and its relationship to obesity and body composition in children.

A) T2 parametric map (right) and high resolution image (left) depicting regions of interest. B) T2 relaxation time in children with obesity and lean controls was obtained for the mediobasal hypothalamus (MBH), putamen and amygdala. Values are adjusted for age. C) Physiological age-related decline is absent for the MBH T2 relaxation time only for children with obesity (r=0.07, p=0.85), not for lean controls (r=−0.76, p=0.02). Correlations between T2 relaxation time in MBH and indices of body fat content by D) BMI Z-score (unadjusted); E) body fat percentage (adjusted for age, sex and puberty); F) visceral fat percentage (unadjusted) and G) android fat percentage (adjusted for age, sex and puberty). P-values were by Spearman's rank correlation coefficient for non-normally distributed variables and linear regression for normally distributed variables.

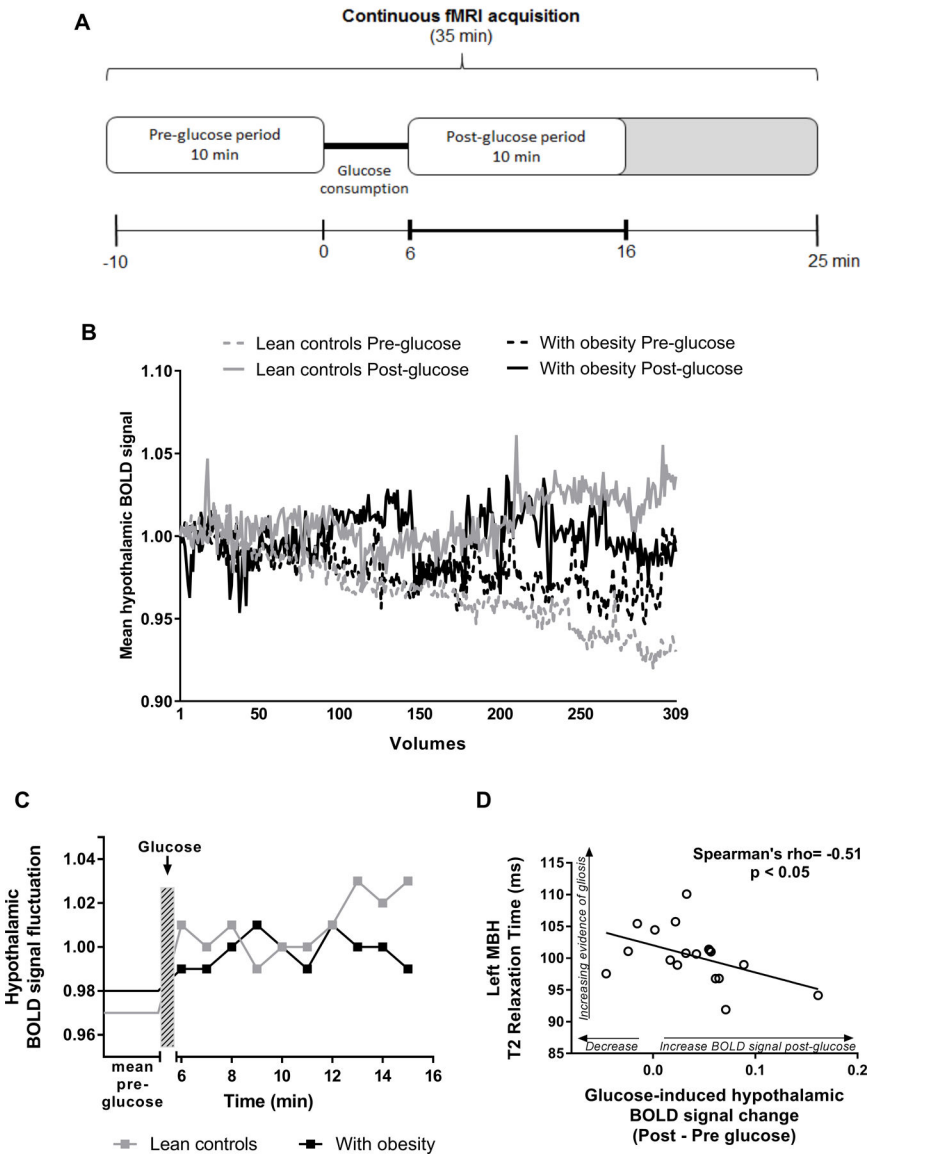

Figure 2. Hypothalamic BOLD signal change in children with obesity and lean controls.

A) The fMRI study protocol included a 10-minute period pre-glucose consumption and a 10-minute post-glucose segment. B) Hypothalamic BOLD signal was normalized to the first volume of each individual's acquisition period (pre- or post-glucose, respectively). Group averages for children with obesity (N=11) and lean controls (N=10) were then calculated and presented for each volume (N=309) acquired during the 10 minutes of continuous monitoring pre- and post-glucose intake. Participants ingested 1.75g/Kg (up to 75g) of glucose. C) Hypothalamic mean pre-glucose BOLD signal does not differ between lean participants and children with obesity. Average per minute of the post-glucose BOLD signal is presented for both groups between 6 and 16 minutes after the beginning of glucose intake. Linear mixed model for repeated measures show no effect of group, time or group*time interaction. D) All participants with complete data were included in a correlational analysis (N=18) of T2 relaxation time with the degree of change in BOLD signal elicited by glucose

ingestion. This was calculated as post- minus pre-glucose, therefore positive numbers represent greater increases in BOLD signal after glucose. Individual left mediobasal hypothalamus (MBH) T2 relaxation times were compared by Spearman's rank correlation coefficient to individual mean glucose-induced hypothalamic BOLD signal change.

Table 1.

General characteristics, blood hormone and cytokines, and T2 relaxation time from quantitative T2 magnetic resonance in children with obesity and lean controls.

	Groups										
	Lean controls					With obesity					P
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	
Anthropometric parameters											
Age, *y*	12	13.3	2.1	9.9	17.1	11	12.9	2.7	10.1	17.4	0.73
Weight Z-score	12	−0.5	0.9	−1.9	1.3	11	2.0	0.6	1.0	2.8	<0.001
Height Z-score	12	−0.3	0.9	−1.9	1.2	11	0.3	1.2	−1.4	2.4	0.16
BMI, kg/m^2	12	18.1	2.4	14.7	22.0	11	29.5	3.2	26.0	35.6	<0.001
BMI Z-score	12	−0.4	0.8	−2.0	1.0	11	2.1	0.3	1.7	2.4	<0.001
Fat mass, *kg*	12	10.6	3.9	6.6	19.7	11	29.1	9.1	19.5	47.9	<0.001
Lean mass, *kg*	12	31.8	8.8	18.1	46.9	11	38.6	12.5	22.6	56.9	0.14
Body fat %[a]	12	24.3	6.6	14.2	36.5	11	42.2	6.8	25.3	51.6	<0.001
Android fat %[b]	12	3.9	1.0	2.7	5.5	11	7.8	0.8	6.5	9.3	<0.001
Gynoid fat %[b]	12	17.4	1.8	14.5	21.2	11	16.7	1.4	14.7	18.5	0.27
Visceral fat, *g*	11	89.5	30.3	30.0	138.0	11	598.9	267.5	236.0	1069.0	<0.001
Visceral fat %[a]	11	1.0	0.5	0.4	2.0	11	2.0	0.5	1.2	2.6	<0.001
Hormonal and inflammatory markers											
Insulin, *mcU/mL*	11	6.3	1.8	4.1	8.9	11	6.2	3.2	4.1	15.3	0.57
Leptin, *ng/mL*	11	4.8	4.9	0.6	16.4	11	33.4	15.5	4.1	55.4	<0.001
Adiponectin, *ng/mL*	11	6709.2	1261.0	5124.0	9742.8	11	4360.9	1714.6	1808.8	6904.1	0.001
IL-10, *pg/mL*	11	0.6	0.2	0.4	0.9	11	0.6	0.1	0.5	0.9	0.42
TNF-α, *pg/mL*	11	1.2	0.5	0.7	2.2	11	2.0	0.7	0.8	3.3	0.007
IL-6, *pg/mL*	11	0.9	0.4	0.4	2.0	11	2.9	1.7	0.4	6.2	0.001
Quantitative T2 MRI											
Left Putamen	9	70.2	2.4	65.2	73.3	11	70.0	2.6	64.4	74.0	0.823
Right Putamen	9	70.7	1.7	68.7	73.9	11	70.3	2.7	66.0	74.6	0.709
Mean Putamen	9	**70.5**	**1.8**	**67.3**	**73.0**	11	**70.1**	**2.5**	**65.2**	**73.4**	**0.749**
Left Amygdala	9	87.5	3.2	84.0	92.7	11	88.2	4.8	80.0	95.5	0.732
Right Amygdala	9	87.9	3.3	85.3	93.7	11	88.2	3.3	82.3	95.3	0.881
Mean Amygdala	9	**87.7**	**3.1**	**84.7**	**92.0**	11	**88.2**	**3.8**	**81.1**	**93.9**	**0.941**
Left MBH	9	96.6	3.7	91.9	101.2	11	102.1	4.0	96.8	110.1	0.005
Right MBH*	9	102.4	5.2	92.9	107.8	11	109.7	6.9	101.5	126.2	0.016
Mean MBH	9	**99.5**	**4.4**	**92.4**	**104.2**	11	**105.9**	**4.2**	**99.7**	**113.8**	**0.004**

N=number of participants; SD=standard deviation; BMI=body mass index;

[a]%=relative to total body mass;

[b]%=relative to total fat mass;IL-6=interleukin-6; IL-10=interleukin-10; TNF-α=tumor necrosis factor-alpha; MRI=Magnetic Resonance Imaging; MBH=Mediobasal hypothalamus;

* Right MBH had longer T2 relaxation time then left MBH for both lean ($t(8)=-7.26$, $p<0.01$) and children with obesity $t(10)=-3.43$, $p=0.01$).