Inverse association between hypothalamic N-acetyl aspartate/creatine ratio and indices of body mass in adolescents with obesity

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Conflict of interest

All authors declare no conflict of interest.

Abbreviations

BES: Binge Eating Scale; BMI: Body Mass Index; CDI: Child Depression Inventory; Cho: total Choline; Cr: Creatine; Glu: Glutamate; Glx: sum of glutamate and glutamine; GPC: glycerophosphocholine; mI: Myoinositol; NAA: N-acetylaspartate; PCh: phosphocholine; YFAS: Yale Food Addiction Scale; 1H-MRS: proton magnetic resonance spectroscopy.

ABSTRACT

Background: Approximately 10% of adolescents worldwide are overweight or obese, hence the urgent and universal need to elucidate possible mechanisms that lead to obesity in the adolescent population.

Objective: To examine hypothalamic metabolism and its relationship with physical development in obese and eutrophic adolescents.

Methods: We performed a case-control study with 115 adolescents between 11 and 18 years of age, to compare obese (BMI z-score ≥2) and non-obese individuals (eutrophic control, BMI z-score ≤1). The following hypothalamic metabolite ratios were examined as primary outcomes: Glutamate/creatine (Glu/Cr), sum of glutamate and glutamine/Cr (Glx/Cr), N-acetylaspartate/Cr (NAA/Cr), Myoinositol/Cr (mI/Cr) and total Choline/Cr (GPC+PCh/Cr),
quantified by magnetic resonance spectroscopy. BMI z-scores, pubertal status, Yale Food Addiction Scale, the Binge Eating Scale, and the Child Depression Inventory were assessed as secondary outcomes. Pearson coefficients ($r$) or nonparametric Spearman correlation ($\rho$) analyses were performed between hypothalamic metabolite ratios and other parameters, such as BMI z-scores, physical development, food habits, depression symptoms and serum protein concentrations (cytokines, hormones and neuropeptides).

**Results:** Adolescents with obesity showed lower hypothalamic NAA/Cr ratio ($0.70 \pm 0.19$) compared to their eutrophic counterparts ($0.84 \pm 0.20$) ($P=0.004$). The NAA/Cr ratio was negatively correlated with BMI z-scores ($r=-0.25$, $P=0.03$) and serum insulin ($\rho=-0.27$, $P=0.04$), C-peptide ($\rho=-0.26$, $P=0.04$), amylin ($r=-0.27$, $P=0.04$), ghrelin ($\rho=-0.30$, $P=0.02$) and neuropeptide Y ($r=-0.27$, $P=0.04$). Also, NAA/Cr ratio was positively correlated with circulating IL-8 levels ($\rho=0.26$, $P=0.04$).

**Conclusions:** High BMI z-scores are associated with lower hypothalamic NAA/Cr ratios. The negative correlations found between NAA/Cr ratio and serum cytokines, hormones and neuropeptides suggest a broad cross-talk linking hormonal imbalance, neurohumoral alterations and hypothalamic function in adolescents with obesity.

**Keywords:** spectroscopy, neuroimaging, adolescent obesity, hypothalamus, brain damage.
INTRODUCTION

Approximately 10% of adolescents worldwide are overweight or obese (1), hence the urgent and universal need to address the consequences of this disease on the physical development of adolescents. Equivocal dietary choices lead to obesity and comorbidities including diabetes, cardiovascular disease, and an increased frequency of cancers (2). Furthermore, global forecasts predict an increasing rate of obesity, which will negatively affect future health and economic policies (3). Obesity in early life is associated with more health problems in adulthood (1); thus, preventing obesity in childhood and adolescence is critical. However, several studies conducted in the United States (1,4) have reported that obesity rates remain high and that no significant improvements were observed between the periods of 2003-2003 and 2011-2012 (4). In Brazil, the most recent nationwide survey conducted in 2021 by the Ministry of Health estimated that 6.4 million children are overweight. Of these, over 3.1 million have already evolved to obesity (based on the BMI of children in primary Health Care System records). In other words, 28% of Brazilian children are overweight and 13.2% are obese (5,6). Interestingly, significant differences were found among different socioeconomic classes, with underprivileged children showing lower obesity rates than children in higher social classes (2.5% vs. 10.6%, respectively)(7). It is well-known that diet plays a prominent role in the etiology of obesity, and appetite control and eating behaviors involve a complex network of neural systems. However, less is known about the role of the adolescent hypothalamus in these processes.

The hypothalamus is associated with important basic functions such as the control of homeostasis, regulation of energy balance, reproduction, and food intake (8). This brain region is highly sensitive to peripheral metabolic mediators and integrates various neural, hormonal, and nutritional signals (9). Thus, the hypothalamus is critical in regulating metabolism, appetite, weight and body composition (10). Studies with magnetic resonance
imaging (MRI) in obese adults reported decreased signal intensity in T2-weighted images of the mediobasal hypothalamus, suggesting gliosis (11), as well as decreased hypothalamic connectivity, as measured by diffusion tensor imaging (12). However, most of the literature focuses solely on other brain structures, like the hippocampus.

The hippocampus and the hypothalamus play a similar role in appetite control (13). Previous studies suggest that hippocampal neurons rely on neurohormonal signals to orchestrate a response to achieve energy balance through adaptive behavioral outcomes (14). Several rodent studies showed that excessive caloric intake via diets high in lipids and sugars reduces hippocampal function, plasticity and neurogenesis (14–16). In humans, previous studies reported an inverse relationship between BMI and hippocampal N-acetylaspartate (NAA), indicating that a higher BMI is associated with lower hippocampal NAA concentration levels (13). Recent proton magnetic resonance spectroscopy (1H-MRS) studies also show that a higher BMI is associated with decreased content of neurochemical markers of neuronal integrity (N-acetylaspartate) in several brain regions like the hippocampus (17). Moreover, in adults with intact cognitive function, obesity is associated with altered cerebral neurochemical profiles, increased myoinositol (mI) levels and decreased NAA/Cr and Glu (glutamate)/Cr ratios in the hippocampal region (18,19).

Although previous research suggests an important association between hippocampal composition and obesity, little is known about the association between hypothalamic metabolism (Glu/Cr, Glx/Cr, NAA/Cr, mI/Cr, and GPC+PCh/Cr ratios), body weight, physical development and food habits in adolescents. Therefore, our aim was to assess hypothalamic metabolism in adolescent obesity.

We hypothesized that adolescent obesity would be associated with a decrease in the NAA/Cr ratio in the hypothalamus, a sign of synaptic loss and neurodegeneration. Furthermore, we
investigated the association between hypothalamic metabolism and BMI, development and food habits in obese and eutrophic adolescents.

METHODS

Study design
This is a case-control study to evaluate the association of hypothalamic metabolism, body weight, physical development and food habits, comparing adolescents with obesity and their eutrophic counterparts, of both sexes. This study is described per the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines (20).

Ethics
The Institutional Review Board of the Santa Casa de São Paulo Hospital approved the study (CAAE: 24552413.2.0000.5479). Informed assent and consent forms were signed by adolescents and their parents/guardians, respectively, before the implementation of any study protocol.

Participants
We collected data from the Childhood Obesity Outpatient Clinic of the Santa Casa de São Paulo. Participant recruitment started in May of 2015, and follow-up ended in September of 2016. We included adolescents between the ages of 11 and 18; the eutrophic group (control) had a BMI z-score ≤ 1 and the obesity group had a BMI z-score ≥ 2. We excluded subjects presenting neurological disorders, head trauma, or ferromagnetic objects in the body including orthodontic appliances that could affect safety and/or the quality of the magnetic resonance imaging exam. We excluded subjects with substance dependence or abuse, as well any psychiatric disorders diagnosed using the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS) questionnaire (21) (see flow chart, Figure 1).
Anthropometric variables

Systolic and diastolic blood pressure were measured with a sphygmomanometer. Age at menarche was recorded for female participants. Pubertal status was measured using the Tanner staging system, a scale of physical development in children, adolescents, and adults. The scale defines physical measurements of development based on external primary and secondary sex characteristics, such as the size of breasts and genitals, testicular volume and the development of pubic hair (22). Tanner staging was not performed via physical examination to prevent adolescents from feeling uncomfortable; instead, participants were asked to identify figures that best resembled their pubic development stage.

Psychological and socio-economic assessments

The Yale Food Addiction Scale (YFAS) and the Binge Eating Scale (BES) were assessed as described by Simoes et al. (23). Child Depression Inventory (CDI) was assessed considering a 27-item self-reported measure that assesses the presence of subsyndrome depressive symptoms in children and adolescents from ages 7 to 17 (24). The “Brazilian Economic Classification” was employed to classify participants according to socioeconomic strata (25).

Image acquisition

Magnetic Resonance Spectroscopy (MRS) was performed using a 3.0T whole body magnet (Intera Achieva, Philips, Best, the Netherlands) in the Department and Institute of Radiology at the University of São Paulo (InRad HC-FMUSP) between 7 and 7:30 a.m (the patients were in a fasted state during the exam). The Point Resolved single voxel (PRESS) technique was used with a TE/TR of 35/4000 ms and 160 repetitions. The nominal voxel size of the spectrum was $2 \times 2 \times 2 \text{ cm}^3$ and was located at the height of the hypothalamus, with four saturation bars placed within the limits of the water-voxel overlap with NAA-voxel to reduce the effect of the chemical shift (Supplementary Figure 1).
MRS Quantification

LCModel (version 6.3) was adopted to quantify metabolite levels of Glutamate (Glu), sum of glutamate and glutamine (Glx), N-acetylaspartate (NAA), Myoinositol (mI), total Choline (Cho) as the sum of glycerophosphocholine (GPC) and phosphocholine (PCh), and Creatine (Cr) (26). An unsuppressed water signal was used as an internal reference. To ensure the accuracy of the measurements obtained, only metabolite results with Cramer–Rao lower bound (CRLB) values of less than 20% were considered (27). In the same way, spectra of low spectral resolution (FWHM > 0.1ppm) and a signal to noise ratio below 5 were excluded from the analysis (Supplementary Table 1). We report metabolite concentrations as ratio to Cr, since absolute values are usually considered less reliable, as they are more susceptible to partial volume effects than ratios over Cr.

Statistical methods

Data are expressed as mean ± SD (standard deviation). Categorical variables were analyzed using Chi-squared tests. Mammary development and gonad development were analyzed within the respective gender categories. Normality was checked using the Kolmogorov–Smirnov test in continuous variables and comparisons were conducted through analysis of variance using Unpaired T tests (parametric) or Mann-Whitney (nonparametric) tests, when appropriate. Pearson (parametric coefficient) or Spearman correlations (nonparametric coefficient) were conducted to evaluate the association between spectroscopy metabolite ratios and BMI z-scores, BES, YFAS and CDI. The data obtained in the present study were correlated with circulating levels of cytokines, hormones and neuropeptides measured in the same cohort by Simoes et al. (23,28). Results are reported as predicted means with 95% confidence intervals and significance was considered at $P$-value < 0.05. All analyses were performed using Graphpad Prism 7.0.
RESULTS

Participants

Table 1 reports the characteristics of the entire cohort. A total of 115 subjects, on average 13.9 ± 1.93 years-old, of which 52.2% females, were divided in two groups: eutrophic group (control) with BMI z-scores ≤1 and an obesity group with BMI z-scores ≥2. Potential confounders such as age and sex presented no differences in the distribution between groups (p>0.05). Adolescents with obesity presented higher systolic blood pressure (SBP) (122 ± 14.3 mmHg) compared to their eutrophic counterparts (108 ± 8.44 mmHg) (P< 0.001). No differences were found regarding pubic hair development (P=0.35), mammary development (P=0.17), or gonad development (P=0.07). Average menarche age among females was 11.6 ± 1.19 years. Adolescents with obesity showed higher scores on The Yale Food Addiction Scale (YFAS, P=0.02) and the Binge Eating Scale (BES, P=0.001), indicating addiction to high-fat and/or high-sugar foods and eating disorders, respectively, when compared to normal weight controls.

Hypothalamic metabolite levels in adolescents

Out of a total of 115 spectra obtained, only 74 spectra (37 per group) fulfilled the established quality criteria and were considered for further analysis. The high number of excluded low-quality spectra (n=41) is related to the location of the hypothalamus, which is very difficult to shim (homogenization of the B₀ magnetic field) and will often lead to a non-optimal equipment adjustment. MRS quality parameters, such as the frequency width at half maximum (FWHM), signal to noise ratio (SNR) and Cramér-Rao Lower Bounds (CRLB) for each metabolite, were calculated (Supplementary Table 1) and no differences in terms of MRS quality were observed between groups.
Exploratory analysis considering the entire cohort demonstrated that adolescents with obesity presented lower N-acetylaspartate/creatine ratios (NAA/Cr) relative to eutrophic adolescents ($P = 0.004$) (Table 2). Glu/Cr, Glx/Cr, mI/Cr and GPC+PCh/Cr ratios did not present differences between groups.

Significant differences in hypothalamic levels of NAA, as well as the BMI z-scores of the entire cohort were considered for correlation analyses, which showed significant negative linearity between BMI z-scores with NAA/Cr ratio ($P=0.03$), indicating that lower hypothalamic levels of NAA are associated with increased BMI z-scores (Figure 2). However, no correlations were found between hypothalamic NAA content and physical development, food habits or depression symptoms (Supplementary Table 2).

Relationship between circulating factors and hypothalamic metabolites

To evaluate the cross-talk between serum protein concentrations and central metabolites, correlation analyses were performed for NAA/Cr and circulating cytokines, hormones or neuropeptides (Table 3). There was a negative correlation between the NAA/Cr ratio and insulin ($\rho = -0.27, P = 0.04$; Figure 3A), C-peptide ($\rho = -0.26, P = 0.04$; Figure 3B), amylin ($r = -0.27, P = 0.04$; Figure 3C) and ghrelin ($\rho = -0.30, P = 0.02$; Figure 3D). Furthermore, the NAA/Cr ratio was negatively correlated with neuropeptide concentration [neuropeptide Y ($r = -0.27, P = 0.04$; Figure 3E)] and positively correlated with cytokine levels [IL-8 ($\rho = 0.26$, $P = 0.04$; Figure 3F)]. No other correlations were detected between NAA/Cr and circulating factors.
DISCUSSION

To the best of our knowledge, this is the first study evaluating the relationship of hypothalamic metabolite composition (Glu/Cr, Glx/Cr, NAA/Cr, mI/Cr, and GPC+PCh/Cr ratios) with body weight, physical development, and food habits in adolescents. Higher BMI z-scores were associated with lower NAA/Cr ratios in the hypothalamus, a brain area that has been deemed crucial for weight homeostasis.

The NAA/Cr ratio is considered an indicator of functional integrity, neuronal density, and overall brain activity (29). Consequently, a decrease in the NAA/Cr ratio is considered a sign of synaptic loss and neurodegeneration (29). NAA presence is high in neurons, since it is an active component of various processes including myelination, myelin repair, lipid metabolism, osmoregulation, and neuronal signaling (30). NAA is also as a key component of the regulation of oligodendrocyte metabolism during brain development and in situations involving brain damage. The findings thus suggest that obese adolescents may be at risk of developing impairments in these processes, with possible long-term consequences.

The finding that high BMI z-scores were associated with lower NAA/Cr corroborates studies reporting that the pathogenesis of obesity involves increased adiposity, which is linked to progressively compromised weight loss capacity (31,32). The impaired connectivity and hypothalamic gliosis observed among obese individuals may possibly contribute to cognitive dysfunction, weight gain, and metabolic disease (33–35). The literature stresses that decreased NAA/Cr ratios may function as a marker for the reduction of neuronal dysfunction and dopaminergic neurotransmission, which are involved in the regulation of memory, attention, learning, and executive functioning (36,37). Spectroscopic studies in obese adolescents demonstrated lower levels of NAA and choline (Cho) in relation to their eutrophic counterparts, but no significant differences were observed in Creatine (Cr), NAA/Cr, or Cho/Cr in the frontal lobe and hippocampus (38). These findings are noteworthy,
as alterations frequently associated with obesity, such as insulin resistance, may also be related to lower NAA (39). Similarly, our data demonstrate a negative correlation between NAA/Cr and insulin. Furthermore, C-peptide (released during insulin cleavage) and amylin were also negatively correlated with NAA/Cr, indicating that increased circulating hormone concentrations could contribute to the severity of hypothalamic impairment (lower NAA/Cr ratios) in higher z-score patients. Nonetheless, the specific mechanism driving such a disruption is unknown (40,41). We hypothesize that insulin resistance leads to increased levels of circulating cytokines, with the potential to cross the blood-brain barrier and modulate NAA concentrations. The same can be postulated in regard to augmented levels of circulating glucose (18). We also describe a positive correlation between IL8 and NAA/Cr ratios.

The relevance of the present study resides in demonstrating that patients with higher BMI z-scores also show signs of altered hypothalamic metabolism, as inferred by lower NAA/Cr ratios. Considering the hypothalamus as the major center of appetite control and metabolism regulation, the correlations found between NAA/Cr and serum cytokines, hormones and neuropeptides may suggest an intricate cross-talk, linking hormonal imbalance, neurohumoral alteration, and hypothalamic function in adolescents with obesity.

A previous report (42) suggests that lower metabolite ratios associated with higher BMI may not reflect real physiological changes, but rather lower quality of the spectrum in the presence of thicker layers of subcutaneous fat. In our study we showed that MRS quality for both groups were comparable, reinforcing the idea that our findings represent real metabolic changes. In agreement with this, a recent in vitro study also showed that the presence of a fat layer does not affect MRS quantification (43).

While our study fills a gap in the literature, it does has limitations that are usually associated with an observational design. Firstly, our metabolite measurements were not validated
through agreement across different measurements, thus introducing potential measurement bias. Secondly, we did not follow the participants longitudinally to evaluate whether these associations could be modified over time. Thirdly, the described correlations may be considered relatively weak. Nevertheless, the novelty consists in a comprehensive and concomitant analysis of variables within the same population, thus achieving clinical relevance. Finally, although our sample was one of the largest in the literature, the final number of participants can be deemed relatively small. This fact can be explained by the rigid quality control applied to our MRS data, which left us with only 74 samples, out of 115, considered acceptable for further data analysis. Future studies should take this reduction into consideration when planning data collection, as image quality can be affected by the surrounding body tissue and cerebrospinal fluid.

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Author contribution

TN and ES: conception and design of the study, collection of samples, molecular experiments, statistical analysis and manuscript writing. ELBC, PB, NC, FD, MGMM, VHOO, TZSO, DACV, GB and CK: collection of samples, data analyses and manuscript revision. JCL: molecular experiments, data analyses and manuscript revision. MS: supervision of the study and manuscript writing. MCO and RRU: conception, design and supervision of the study and manuscript writing. All authors have read and approved the final manuscript.
Data availability

Data described in the manuscript, code book and analytic code will be made available upon request to the corresponding author Estefania.simoesfer@gmail.com
REFERENCES

1. Collins CE, Watson J, Burrows T. Measuring dietary intake in children and adolescents in the context of overweight and obesity. *Int J Obes (Lond)* (2010) 34:1103–15. doi:10.1038/ijo.2009.241

2. Aicr, WCRF. Diet, Nutrition, Physical Activity and Cancer: a Global Perspective A summary of the Third Expert Report. (2018). Available at: http://gco.iarc.fr/today

3. Gortmaker SL, Swinburn BA, Levy D, Carter R, Mabry PL, Finegood DT, Huang T, Marsh T, Moodie ML. Changing the future of obesity: science, policy, and action. *Lancet (London, England)* (2011) 378:838–47. doi:10.1016/S0140-6736(11)60815-5

4. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of Childhood and Adult Obesity in the United States, 2011-2012. *JAMA* (2014) 311:806. doi:10.1001/jama.2014.732

5. Fradkin C, Yunes MAM. Childhood obesity in Brazil: lessons to be learned from the Northern Hemisphere. *Educa Ciência e Cult* (2014) 19:117–122. doi:10.18316/1869

6. Ferreira CM, Reis ND dos, Castro A de O, Höfelmann DA, Kodaira K, Silva MT, Galvao TF. Prevalence of childhood obesity in Brazil: systematic review and meta-analysis. *J Pediatr (Rio J)* (2021) 97:490–499. doi:10.1016/J.JPED.2020.12.003

7. de Oliveira AMA, Cerqueira E de MM, de Oliveira AC. [Prevalence of overweight and childhood obesity in Feira de Santana-BA: family detection vs. clinical diagnosis]. *J Pediatr (Rio J)* (2003) 79:325–8. doi:10.2223/jped.1052

8. Gabriela Pop M, Crivii C, Opincariu I. “Anatomy and Function of the Hypothalamus,” in *Hypothalamus in Health and Diseases* (IntechOpen). doi:10.5772/intechopen.80728

9. Schwartz MW, Porte D. Diabetes, obesity, and the brain. *Science* (2005) 307:375–9. doi:10.1126/science.1104344
10. Toda C, Santoro A, Kim JD, Diano S. POMC Neurons: From Birth to Death. *Annu Rev Physiol* (2017) **79**:209–236. doi:10.1146/annurev-physiol-022516-034110

11. Kreutzer C, Peters S, Schulte DM, Fangmann D, Türk K, Wolff S, van Eimeren T, Ahrens M, Beckmann J, Schafmayer C, et al. Hypothalamic Inflammation in Human Obesity Is Mediated by Environmental and Genetic Factors. *Diabetes* (2017) **66**:2407–2415. doi:10.2337/db17-0067

12. Puig J, Blasco G, Daunis-I-Estadella J, Molina X, Xifra G, Ricart W, Pedraza S, Fernández-Aranda F, Fernández-Real JM. Hypothalamic damage is associated with inflammatory markers and worse cognitive performance in obese subjects. *J Clin Endocrinol Metab* (2015) **100**:E276–81. doi:10.1210/jc.2014-2682

13. Coplan JD, Fathy HM, Abdallah CG, Ragab SA, Kral JG, Mao X, Shungu DC, Mathew SJ. Reduced hippocampal N-acetyl-aspartate (NAA) as a biomarker for overweight. *NeuroImage Clin* (2014) **4**:326–35. doi:10.1016/j.nicl.2013.12.014

14. Davidson TL, Kanoski SE, Schier LA, Clegg DJ, Benoit SC. A potential role for the hippocampus in energy intake and body weight regulation. *Curr Opin Pharmacol* (2007) **7**:613–6. doi:10.1016/j.coph.2007.10.008

15. Monteggia LM, Barrot M, Powell CM, Berton O, Galanis V, Gemelli T, Meuth S, Nagy A, Greene RW, Nestler EJ. Essential role of brain-derived neurotrophic factor in adult hippocampal function. *Proc Natl Acad Sci U S A* (2004) **101**:10827–32. doi:10.1073/pnas.0402141101

16. Kanoski SE, Meisel RL, Mullins AJ, Davidson TL. The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat. *Behav Brain Res* (2007) **182**:57–66. doi:10.1016/j.bbr.2007.05.004

17. Kaur S, Birdsill AC, Steward K, Pasha E, Kruzliak P, Tanaka H, Haley AP. Higher visceral fat is associated with lower cerebral N-acetyl-aspartate ratios in middle-aged
18. Gazdzinski S, Millin R, Kaiser LG, Durazzo TC, Mueller SG, Weiner MW, Meyerhoff DJ. BMI and Neuronal Integrity in Healthy, Cognitively Normal Elderly: A Proton Magnetic Resonance Spectroscopy Study. *Obesity* (2010) **18**:743–748. doi:10.1038/oby.2009.325

19. Gonzales MM, Tarumi T, Eagan DE, Tanaka H, Vaghasia M, Haley AP. Indirect effects of elevated body mass index on memory performance through altered cerebral metabolite concentrations. *Psychosom Med* (2012) **74**:691–8. doi:10.1097/PSY.0b013e31825ff1de

20. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Prev Med (Baltim)* (2007) **45**:247–51. doi:10.1016/j.ypmed.2007.08.012

21. Young ME, Bell ZE, Fristad MA. Validation of a Brief Structured Interview: The Children’s Interview for Psychiatric Syndromes (ChIPS). *J Clin Psychol Med Settings* (2016) **23**:327–340. doi:10.1007/s10880-016-9474-7

22. Tanner J. Growth at adolescence. springfield, illinois. (1962) Available at: http://www.garfield.library.upenn.edu/classics1986/A1986C968000001.pdf

23. Simoes E, Correia-Lima J, Calfat EL de B, Otani TZ dos S, Vasques DAC, Otani VHO, Bertolazzi P, Kochi C, Seelaender M, Uchida RR. Sex-Dependent Dyslipidemia and Neuro-Humoral Alterations Leading to Further Cardiovascular Risk in Juvenile Obesity. *Front Nutr* (2021) **7**:1–10. doi:10.3389/fnut.2020.613301

24. Figueras Masip A, Amador-Campos JA, Gómez-Benito J, del Barrio Gándara V. Psychometric properties of the Children’s Depression Inventory in community and clinical sample. *Span J Psychol* (2010) **13**:990–9. doi:10.1017/s1138741600002638
25. Kamazura W and Mazzon JA., Critérios de estratificação e comparação de classificadores socioeconômicos no Brasil. Rev Adm Empres (2016) 56:55–70. doi:10.1590/S0034-759020160106

26. Provencher SW. Automatic quantitation of localized in vivo 1H spectra with LCMModel. NMR Biomed (2001) 14:260–4. doi:10.1002/nbm.698

27. Kreis R. Issues of spectral quality in clinical 1H-magnetic resonance spectroscopy and a gallery of artifacts. NMR Biomed (2004) 17:361–81. doi:10.1002/nbm.891

28. Simoes E, Correia-Lima J, Sardas L, Storti F, dos Santos Otani TZ, Vasques DAC, Otani VHO, Bertolazzi P, Kochi C, Seelaender M, et al. Sex dimorphism in inflammatory response to obesity in childhood. Int J Obes (2021) 45:879–887. doi:10.1038/s41366-021-00753-1

29. Calderón-Garcidueñas L, Mora-Tiscareño A, Melo-Sánchez G, Rodríguez-Díaz J, Torres-Jardón R, Styner M, Mukherjee PS, Lin W, Jewells V. A Critical Proton MR Spectroscopy Marker of Alzheimer’s Disease Early Neurodegenerative Change: Low Hippocampal NAA/Cr Ratio Impacts APOE ε4 Mexico City Children and Their Parents. J Alzheimer’s Dis (2015) 48:1065–1075. doi:10.3233/JAD-150415

30. Moffett JR, Arun P, Ariyannur PS, Namboodiri AMA. N-Acetylaspartate reductions in brain injury: impact on post-injury neuroenergetics, lipid synthesis, and protein acetylation. Front Neuroenergetics (2013) 5:11. doi:10.3389/fnene.2013.00011

31. Guyenet SJ, Schwartz MW. Regulation of food intake, energy balance, and body fat mass: Implications for the pathogenesis and treatment of obesity. J Clin Endocrinol Metab (2012) 97:745–755. doi:10.1210/jc.2011-2525

32. Berkseth KE, Guyenet SJ, Melhorn SJ, Lee D, Thaler JP, Schur EA, Schwartz MW. Hypothalamic gliosis associated with high-fat diet feeding is reversible in mice: a combined immunohistochemical and magnetic resonance imaging study.
33. Thaler JP, Yi C-X, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, et al. Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* (2012) **122**:153–62. doi:10.1172/JCI59660

34. Dorfman MD, Thaler JP. Hypothalamic inflammation and gliosis in obesity. *Curr Opin Endocrinol Diabetes Obes* (2015) **22**:325–30. doi:10.1097/MED.0000000000000182

35. Schur EA, Melhorn SJ, Oh S-K, Lacy JM, Berkseth KE, Guyenet SJ, Sonnen JA, Tyagi V, Rosalynn M, De Leon B, et al. Radiologic evidence that hypothalamic gliosis is associated with obesity and insulin resistance in humans. *Obesity (Silver Spring)* (2015) **23**:2142–8. doi:10.1002/oby.21248

36. Wiguna T, Guerrero APS, Wibisono S, Sastroasmoro S. Effect of 12-week administration of 20-mg long-acting methylphenidate on Glu/Cr, NAA/Cr, Cho/Cr, and mI/Cr ratios in the prefrontal cortices of school-age children in Indonesia: a study using 1H magnetic resonance spectroscopy (MRS). *Clin Neuropharmacol* (2012) **35**:81–5. doi:10.1097/WNF.0b013e3182452572

37. Jessen F, Fingerhut N, Sprinkart AM, Kühn KU, Petrovsky N, Maier W, Schild HH, Block W, Wagner M, Träber F. N-acetylaspartylglutamate (NAAG) and N-acetylaspartate (NAA) in patients with schizophrenia. *Schizophr Bull* (2013) **39**:197–205. doi:10.1093/schbul/sbr127

38. Sun J, Chen P, Bi C. 1H-MRS technique and spectroscopic imaging LCModel based adolescent obese metabolic syndrome research. *Multimed Tools Appl* (2017) **76**:19491–19505. doi:10.1007/s11042-015-3191-3

39. Baslow MH. N-Acetylaspartate in the Vertebrate Brain: Metabolism and Function. *Neurochem Res* (2003) **28**:941–953. doi:10.1023/A:1023250721185
40. Karczewska-Kupczewska M, Tarasów E, NikoŁajuk A, Stefanowicz M, Matulewicz N, Otziomek E, Górska M, Straczkowski M, Kowalska I. The effect of insulin infusion on the metabolites in cerebral tissues assessed with proton magnetic resonance spectroscopy in young healthy subjects with high and low insulin sensitivity. *Diabetes Care* (2013) **36**:2787–2793. doi:10.2337/dc12-1437

41. Heurling K, Johansson E, Leuzy A. “Disturbances in brain energy metabolism in insulin resistance and diabetes and Alzheimer’s disease — Learnings from brain imaging biomarkers,” in *International Review of Neurobiology* (Academic Press Inc.), 111–130. doi:10.1016/bs.irn.2020.02.011

42. Mon A, Abé C, Durazzo TC, Meyerhoff DJ. Effects of fat on MR-measured metabolite signal strengths: implications for in vivo MRS studies of the human brain. *NMR Biomed* (2013) **26**:1768–1774. doi:10.1002/nbm.3016

43. Kyathanahally SP, Fichtner ND, Adalid V, Kreis R. Does superficial fat affect metabolite concentrations determined by MR spectroscopy with water referencing? *NMR Biomed* (2015) **28**:1543–1549. doi:10.1002/nbm.3419
Table 1. Anthropometrics, psychological and socio-economic variables of the entire cohort

| Characteristics                  | Eutrophic<sup>2</sup> <br> n=59 | Obese<sup>3</sup> <br> n=56 | Missing values <br> (n) | P-value  |
|----------------------------------|---------------------------------|----------------------------|------------------------|----------|
| Age (years)                      | 14.2 (± 1.91)                  | 13.6 (± 1.91)              | 0                      | 0.09     |
| Sex (Male/Female)                | 26/33                           | 29/27                      | 0                      | 0.41     |
| Blood pressure (mm Hg)           |                                 |                            |                        |          |
| Systolic                         | 107 (± 8.44)                    | 122 (± 14.3)               | 11                     | < 0.001  |
| Diastolic                         | 74.2 (± 10.4)                   | 76.3 (± 14.0)              | 12                     | 0.17     |
| Age at menarche (years)          | 11.7 (± 1.27)                   | 11.5 (± 1.12)              | 7                      | 0.52     |
| Mammary development              |                                 |                            |                        | 0.17     |
| Stage 2                          | 3 (10%)                         | 1 (4 %)                    | 7                      |          |
| Stage 3                          | 11 (38 %)                       | 8 (33 %)                   |                        |          |
| Stage 4                          | 13 (45%)                        | 8 (33%)                    |                        |          |
| Stage 5                          | 2 (7 %)                         | 7 (29%)                    |                        |          |
| Gonad development                |                                 |                            |                        | 0.07     |
| Stage 1                          | 0 (0 %)                         | 1 (5 %)                    | 13                     |          |
| Stage 2                          | 3 (13 %)                        | 5 (26 %)                   |                        |          |
| Stage 3                          | 10 (44%)                        | 3 (16%)                    |                        |          |
| Stage 4                          | 5 (22 %)                        | 9 (47%)                    |                        |          |
| Stage 5                          | 5 (22%)                         | 1 (5 %)                    |                        |          |
| Pubic hair development           |                                 |                            |                        | 0.35     |
| Stage 1                          | 3 (6%)                          | 0 (0 %)                    | 20                     |          |
| Stage 2                          | 8 (15%)                         | 7 (16%)                    |                        |          |
| Stage 3                          | 20 (39%)                        | 13 (30%)                   |                        |          |
| Stage 4                          | 16 (31%)                        | 15 (35%)                   |                        |          |
| Stage 5                          | 5 (10%)                         | 8 (19%)                    |                        |          |
| Psychological assessments<sup>4</sup> |                             |                            |                        |          |
| CDI                              | 10.3 (± 5.44)                   | 10.1 (± 5.32)              | 0                      | 0.85     |
| YFAS                             | 1.67 (± 1.89)                   | 2.29 (± 1.75)              | 0                      | 0.02     |
| BES                              | 6.81 (± 6.17)                   | 10.3 (± 6.49)              | 0                      | 0.001    |
| Socio-economic classification    |                                 |                            |                        | 0.87     |
| Upper class                      | 1 (2%)                          | 2 (4 %)                    | 0                      |          |
| Middle class                     | 31 (53%)                        | 28 (50 %)                  |                        |          |
| Lower class                      | 27 (46%)                        | 26 (46%)                   |                        |          |

<sup>1</sup> Data presented as Mean (± SD). Significance between the groups was tested using chi-squared test for categorical variables and Unpaired T test (systolic blood pressure, age at menarche and CDI) or Mann-Whitney test (age, diastolic blood pressure, YFAS and BES) for continuous variables.

<sup>2</sup> Eutrophic participants presented BMI z-scores ≤1.

<sup>3</sup> Obese patients presented BMI z-scores ≥2.

<sup>4</sup> BES: Binge Eating Scale score; CDI: Children Depression Inventory score; YFAS: Yale Food Addiction scale.
Table 2. Results of proton Magnetic Resonance Spectroscopy in the hypothalamus of the entire cohort\(^1\).

| Ratios\(^2\) | Eutrophic\(^3\) \(n=37\) | Obese\(^4\) \(n=37\) | \(P\)-value |
|--------------|-----------------|-----------------|-------------|
| Glu/Cr       | 1.05 (± 0.17)   | 1.03 (± 0.32)   | 0.85        |
| Glx/Cr       | 1.73 (± 0.36)   | 1.78 (± 0.41)   | 0.59        |
| NAA/Cr       | 0.84 (± 0.20)   | 0.70 (± 0.19)   | 0.004       |
| mI/Cr        | 0.96 (± 0.18)   | 0.99 (± 0.16)   | 0.49        |
| GPC+PCh/Cr   | 0.34 (± 0.04)   | 0.33 (± 0.03)   | 0.19        |

\(^1\) Data presented as Mean (± SD). Significance between the groups was tested using Unpaired T test.
\(^2\) Cr: Creatine; Glu: Glutamate; Glx: Sum of glutamate and glutamine; GPC+PCh: sum of glycerophosphocholine (GPC) and phosphocholine (PCh); mI: Myo-inositol; NAA: N-acetylaspartate.
\(^3\) Eutrophic participants presented BMI z-scores ≤1.
\(^4\) Obese patients presented BMI z-scores ≥2.
Table 3. Serum protein levels and NAA/Cr ratio correlations within the entire cohort

|                      | NAA/Cr |          |          |
|----------------------|--------|----------|----------|
|                      | r      | rho      | P-value  |
| Serum hormones²      |        |          |          |
| Insulin              | -0.27  | 0.04     |          |
| Leptin               | -0.07  | 0.61     |          |
| C-peptide            | -0.26  | 0.04     |          |
| Amylin               | -0.27  | 0.04     |          |
| Glucagon             | -0.19  | 0.15     |          |
| GLP-1                | -0.11  | 0.43     |          |
| GIP                  | -0.12  | 0.36     |          |
| Ghrelin              | -0.30  | 0.02     |          |
| Serum neuropeptides³ |        |          |          |
| α-MSH                | 0.26   | 0.08     |          |
| β-Endorphin          | 0.18   | 0.22     |          |
| Neurotensin          | 0.16   | 0.24     |          |
| Oxytocin             | 0.13   | 0.36     |          |
| Orexin               | 0.13   | 0.36     |          |
| MCH                  | 0.04   | 0.75     |          |
| NPY                  | -0.27  | 0.04     |          |
| Serum cytokines⁴     |        |          |          |
| L1β                  | 0.07   | 0.59     |          |
| IL6                  | 0.08   | 0.57     |          |
| IL8                  | 0.26   | 0.04     |          |
| IL10                 | 0.06   | 0.66     |          |

¹ Significant differences were tested using Pearson coefficients (r) or nonparametric Spearman correlation (rho) within the entire cohort [euthrophic (n=37) and obese (n=37)].
² GIP: gastric inhibitory polypeptide; GLP-1: glucagon-like peptide-1.
³ MCH: melanin-concentrating hormone; α-MSH: α-Melanocyte-stimulating hormone; NPY: neuropeptide Y.
⁴ IL: Interleukin.
**Figure 1.** Flow chart of the MRS study
Figure 2. BMI Z-score correlations to NAA/Cr ratio within the entire cohort

Significant differences were tested using Pearson coefficients ($r$) within the entire cohort [eutrophic ($n=37$) and obese ($n=37$)]. Cr: Creatine; NAA: N-acetylaspartate; $P$: $P$-value; $r$: correlation coefficient.
Figure 3. NAA/Cr ratio, cytokines, hormones and neuropeptides significant correlations within the entire cohort.

A. Serum insulin and hypothalamus NAA/Cr ratio correlation; B. Serum C-peptide and hypothalamus NAA/Cr ratio correlation; C. Serum amylin and hypothalamus NAA/Cr ratio correlation; D. Serum ghrelin and hypothalamus NAA/Cr ratio correlation; E. Serum neuropeptide Y and hypothalamus NAA/Cr ratio correlation; F. Serum IL8 and NAA/Cr Ratio correlation. Significant differences were tested using Pearson coefficients ($r$) or nonparametric Spearman correlation ($rho$) within the entire cohort [eutrophic ($n=37$) and obese ($n=37$)]. Significance was considered at p-value <0.05.