Diminished insulin sensitivity is associated with altered brain activation to food cues and with risk for obesity – implications for individuals born small for gestational age

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Abstract: While classically linked to memory, the hippocampus is also a feeding behavior modulator due to its multiple interconnected pathways with other brain regions and expression of receptor for metabolic hormones. Here we tested whether variations in insulin sensitivity would be correlated with differential brain activation following exposure to palatable food cues, as well as with variations in implicit food memory in a cohort of healthy adolescents, some of whom were born small for gestational age (SGA). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was positively correlated with activation in the cuneus, and negatively correlated with activation in the middle frontal lobe, superior frontal gyrus and precuneus when presented with palatable food images versus non-food images in healthy adolescents. Additionally, HOMA-IR and insulinemia were higher in participants with impaired food memory. SGA individuals had higher snack caloric density and greater chance for impaired food memory. There was also an interaction between the HOMA-IR and birth weight ratio influencing external eating behavior. We suggest that diminished insulin sensitivity correlates with activation in visual attention areas and inactivation in inhibitory control areas in healthy adolescents. Insulin resistance also associated with less consistency in implicit memory for a consumed meal, which may suggest lower ability to establish a dietary pattern, and can contribute to obesity. Differences in feeding behavior in SGA individuals were associated with insulin sensitivity and hippocampal alterations, suggesting that cognition and hormonal regulation are important components involved in food intake modifications throughout life.

Keywords: Intrauterine growth restriction; Small for gestational age; HOMA-IR index; Feeding behavior; Food memory; MRI.

Abbreviations: BWR: birth weight ratio; MRI: magnetic resonance imaging; fMRI: functional magnetic resonance imaging; HOMA-IR: homeostatic model assessment of insulin resistance; AGA: adequate for gestational age; SGA: small for gestational age; BMI: body mass index; IQ: intelligence quotient; WASI: Wechsler abbreviated scale of intelligence; NEUPSILIN-INF: child brief neuropsychological assessment battery; DEBQ: Dutch eating behaviour questionnaire; GLM: general linear model; FWE: family-wise error; ANOVA: analysis of variance; NPY: neuropeptide Y; AgRP: agouti-related protein.
1. Introduction

While the hippocampus is classically associated with memory, this region is also recognized as a feeding behavior modulator due to its multiple interconnected pathways with other brain regions. There is evidence that memory plays a role in the control of hunger and food intake, as the memory of food consumed is used to process subsequent decisions about what, when and how much to eat [1, 2]. Memory, even without conscious awareness, decreases the impact of recent reward and environmental cues on choices, with enhanced connectivity between the hippocampus and prefrontal cortex contributing to reward based decision-making [3].

The hippocampus is sensitive to signals of hunger and satiety, helping to inhibit the ability of food cues to evoke appetite and eating behavior [2, 4]. Hippocampal damage impairs this food inhibitory control system, leading to an altered food intake and increased risk for obesity [5-7]. The hippocampus is the brain region with the larger expression of insulin receptors [8], and insulin action in in this region is associated with cognitive function [9] [10]. Genetic individual variations in the expression of the insulin receptor gene network in the hippocampus predict risk for Alzheimer’s disease in adults, but most importantly, are associated with variations in cognitive capacity in young, healthy children [11].

Human fMRI studies show a positive correlation between fasting plasma insulin levels and hippocampal activity after stimulation with high-caloric food images, which suggests a link between insulin signaling pathways, hippocampal activation, and eating behavior in humans [12-15]. Hippocampal neighboring gyri (parahippocampal and fusiform gyri) are particularly sensitive to insulin and responsive to visual food cues [16, 17].

These findings corroborate the idea that the hippocampus participates in the identification of external signs of food and that insulin is closely linked with that role of the hippocampus in feeding behavior, even in healthy, non-insulin resistant individuals [18].

Variations in early growth trajectories during development – essentially an insulin-dependent process [19] – are associated with differences in childhood executive function [20]. A clinically relevant entity in this context is poor fetal growth, reflected in being born small for a certain gestational age. Subjects born small for gestational age (SGA) are vulnerable to developing type II diabetes as a consequence of inadequate insulin secretion that begins at birth and is followed by a progressive decrease in insulin sensitivity throughout life [21-27]. Interestingly, and in agreement with the notion that central insulin action modifies cognitive and eating behavior outcomes, SGA individuals demonstrate greater preference for hyperpalatable foods (foods high in energy, fat, sugar or salt/sodium) [28-39], and have a higher incidence of cognitive impairments when compared to the general population [40-50].

Cognitive impairments in individuals born with low birth weight have been associated with a reduced volume in the hippocampus [51-57], a brain region vulnerable to neonatal insults [58-63].

In this study, we tested if variations in insulin sensitivity correlate with hippocampal morphometry and brain activation in response to palatable food images [15], as well as if these variations are associated with the ability to retrieve implicitly learned food choices in adolescents [64]. In agreement with previous findings, we hypothesized that poor insulin sensitivity would also contribute to energy intake imbalance and obesogenic behavior [18].

Being born SGA is the most prevalent and clinically significant feature associated with

variations in insulin sensitivity early in life [65], and this condition is linked to altered
behavior towards palatable foods at different ages, which contributes to their increased risk
for chronic metabolic disease later in life [28-39]. To investigate a potential clinical relevance
of this work, a secondary aim of this study was to understand if the behaviors linked to
variations in insulin sensitivity are also prominent in SGA individuals compared to normal
birth weight adolescents. Considering the metabolic programming effects of poor fetal
growth[27, 66], we also investigated if the birth weight status modified the relationship
between HOMA-IR on the eating behavior outcomes.

2. Materials and Methods

2.1 Sample

Subjects included in this study were recruited as part of a prospective cohort, whose
details can be found elsewhere [67]. Participants were recruited from six schools around the
area of a family health clinic that was part of Hospital de Clínicas de Porto Alegre, Porto
Alegre, Brazil. In 2008, children and adolescents from these schools were invited to
participate in the study, which included psychiatric and nutritional assessments. A total of
242 individuals completed the assessment in 2008 and from this initial group, 75 participated
in a more in-depth re-evaluation that included psychiatric and nutritional assessments, DNA
collection and functional Magnetic Resonance Imaging (fMRI) in 2013/2014. To test our
hypotheses, 52 individuals (31 female, 17±2 years) were evaluated (Supplemental figure 1).

The study was approved by the Institutional Ethics Committee of Hospital de Clínicas
de Porto Alegre and the Research Ethics Committee of Pontifícia Universidade Católica do
Rio Grande do Sul. Ethics approval was based on guidelines for research involving humans
and included Resolution 196/96 from the National Health Council and the Declaration of
Helsinki. Local ethics committee approval and written informed consent (either from the
subjects or from their guardians) were obtained from all participants before entering the
study.

2.2 Measurements

The anthropometric measurements were taken in the morning, in the fasted state, by
trained researchers. Weight and height were measured in duplicate (the average value was
used in analyses) using accurate and calibrated equipment, including a digital platform
balance (Toledo, São Paulo, Brazil) and a vertical stadiometer (Harpenden, Holtain Limited,
Crymych, UK). Body mass index (BMI) was calculated as weight (kg) divided by height
(m2) and used as BMI z-scores.

Blood samples were collected in the morning after fasting for 12 h and the samples
were then centrifuged at 4000 rpm for 10 min. Glucose, was measured using an enzymatic
colorimetric method and insulin levels were measured using chemiluminescence ADVIA
1800 and an ADVIA Centaur insulin assay (Siemens Healthcare Diagnostics Inc., Tarrytown,
NY, USA). Homeostasis Assessment Model-Insulin Resistance Index (HOMA-IR) was
calculated using fasting serum insulin (mU/mL) × fasting serum glucose (mmol/L))/22.5.
Socioeconomic status was based on the subject’s education level, the presence of certain items in the household and the education level of the household’s head occupant according to Brazilian Research Companies Association score points. This scale sorts participants into standardized subgroups labeled from A (highest economic strata) to E (lowest economic strata). Intelligence quotient (IQ) was calculated using the *Wechsler Abbreviated Scale of Intelligence* (WASI) [68]. Phonemic and semantic verbal fluency (number of valid words) was evaluated using the Child Brief Neuropsychological Assessment Battery (NEUPSILIN-INF) [69].

Fetal growth was based on the birth weight ratio (BWR), which is the ratio between the infant’s birth weight and the mean birth weight, sex- and gestational age-specific birth weights for the local population [70]. BWR was categorized into adequate for gestational age (“AGA”, two superior tertiles of the BWR distribution) or small for gestational age (“SGA”, those in the lower tertile).

### 2.2.1 Food choices and feeding behavior

After anthropometric measures and blood collection were completed, participants received a voucher to purchase a snack of their choice at the research center’s cafeteria (Figure 1A). The chosen foods were displayed in a tray and photographed. In a new assessment 6 months later, 4 photos of different snacks were shown to the participants, who were fasted at the time, including the photograph from their own previous food choice. Upon the presentation of these photographs, the participants were asked the following question: “If you could eat now, which one of the following snacks would you choose?”. Choices for both composition (Figure 1B) as well as spatial arrangement (Figure 1C) of the snack chosen were investigated. Our intention in this snack choice test was to evaluate implicit, non-declarative food memory, which is known as a category of mnemonic processes involved in automatic behavior [3], that is linked to decision-making that affects food choices thus modulating eating behavior [71, 72]. Food preference choices are one of the best examples of implicit memory because this behavior is not consciously and intentionally learned, and it is resistant to change [73]. Food intake was estimated using the nutritional composition of the selected snack in the first visit. The quantitative analysis of macro- and micronutrients consumed in the selected snack was calculated using the USDA National Nutrient Database [74]. After completing their chosen snack at the research centre’s cafeteria, the participants completed the Dutch Eating Behaviour Questionnaire (DEBQ) [75, 76] that classified eating behavior into restrained, emotional, and external traits according to the questionnaire’s subscales.

### 2.2.2 Structural and functional MRI

**fMRI acquisition parameters:** Of the 52 participants, 40 were eligible for structural and functional MRI (list of exclusion criteria is presented in supplemental figure 1). Participants were fasted for at least 4h and 30min before MRI acquisition, they received a standard snack consisting of a cereal bar and a box of juice containing a total of 174kcal consisting of 39g carbohydrates (90% of total calories), 0.9g protein (2% of total calories) and 1.6g of fats (8% of total calories).

MRI data were acquired using echo planar imaging sequences with a GE 3-Tesla scanner (GE Healthcare Signa HDxT, Waukesha, WI, USA) equipped with an eight-channel head coil. The following parameters were used for structural images: T1 with voxels in isotropic spatial resolution of 1mm³, 170 contiguous slices and matrix images of 256*256 Mucellini AB, Miguel PM, Dalle Molle R, Rodrigues DM, Machado TD, Reis RS, Toazza R, Salum GA, Bortoluzzi A, Franco AR, Buchweitz A, Barth B, Agranonik M, Nassim M, Meaney MJ, Manfro GG, Silveira PP. Diminished insulin sensitivity is associated with altered brain activation to food cues and with risk for obesity - Implications for individuals born small for gestational age. Appetite. 2022 Feb 1;169:105799. doi: 10.1016/j.appet.2021.105799
with inversion recovery type including TE = 2.18 ms and TR = −6.1 ms, and for functional images: 26 axial slices interspersed with a slice thickness of 4.0 mm and gap of 0.4 mm, FOV of 240 mm X 240 mm and matrix size of 80 X 64, TE = 30 ms, TR= 2.000 ms, and a flip angle of 90°.

fMRI design and stimuli: Our fMRI paradigm was described in detail in a previous publication from our lab[77]. It was adapted from [78], and set to investigate brain activation in response to images of palatable and healthy food and other non-food related items (e.g. a chair). Figures were selected from a database of images [79, 80]. A pilot study including adolescents from the same age range as the current study population was performed to establish which food images were perceived as ‘palatable’ or ‘healthy’. The figures were presented using E-Prime software (version 2, Psychological Software Tools, Pittsburgh, PA, USA), with 3 blocks of approximately 7 min each. Each block included 21 randomized images (seven hyperpalatable foods, seven healthy foods and seven non-food objects). Each food trial consisted of image presentation (4 s) followed by two probe questions: ‘How much do you like the food?’ (5 s) and ‘How much do you want to eat the food now?’ (5 s). Participants had responded, by pressing buttons, to a scale that ranged from 1 (zero) to 4 (very much) using an fMRI compatible button box. Inter-trial interval ranged from 3 to 9 s.

Structural and fMRI data analysis: Data were pre-processed and analyzed with SPM8 (SPM8, http://www.fil.ion.ucl.ac.uk/spm) and implemented in MATLAB (MathWorks, Inc., Natick, MA, United States). For each participant, pre-processing analyses included slice timing correction, realignment of functional time series, co-registration of functional and anatomical images, spatial normalization in Montreal Neurological Institute space, spatial smoothing with an 8mm full-width half-maximum Gaussian smoothing kernel, and high- and low-pass filtering. Data from participants who moved over 4mm during imaging were excluded from analyses (3 subjects).

For the structural MRI, we measured regional hippocampal volumes using the well validated multiple automatically generated templates (MAGeT-Brain, https://github.com/CobraLab/MAGeTbrain), a multi-atlas approach that improves segmentation over regular atlas-based approaches [81]. Our focus on the hippocampus was related to the research question of the relationship between variations in insulin resistance, memory and eating behavior in adolescents [18]. MAGeT-Brain minimizes the number of input atlases needed by creating a template library from a sample of the subject images, differently from the voxel-based morphometry (VMB), which is performed by first spatially normalizing all subjects into a common atlas space [82, 83]. Using MAGeT-Brain, volumes for the hippocampus and its subfields (total hippocampal volume, CA4 and DG, CA2 and CA3, CA1, subiculum and stratum radiatum, lacunosum and moleculare, right and left) were extracted for each participant for statistical comparisons (please see statistical analysis section below).

While the structural analysis was focused on the hippocampus to be aligned with the rationale of the study, a whole brain analysis of the functional responses to palatable food images seemed to be more interesting than focusing only on hippocampal functional responses. Therefore, for subject level analyses from fMRI, a general linear model (GLM) was used to estimate changes in brain region responses using four regressors: palatable food, healthy food, and non-food object images and baseline periods (combined with an ideal homodynamic response curve). A two-level hierarchical model was conducted using multiple
regression model analyses in which the HOMA-IR index was used as a predictor. Whole-brain analysis was conducted to identify the brain areas responses to the presentation of images of hyperpalatable food, healthy food, and non-food objects. Correlations between the HOMA-IR index and brain activation were performed with a family-wise error (FWE) correction for multiple comparisons (pFWEcorr<0.05), using the HOMA-IR index as a continuous variable. In an attempt to disentangle the effect of HOMA-IR from BMI differences, we repeated the analysis using zBMI as the predictor, and residualized HOMA-IR (after removing the shared variance with BMI). All t-maps were calculated for the entire cortical volume. We used XJVIEW 8.14 software (http://www.alivelearn.net/xjview/) to display anatomical locations.

2.3 Statistical analyses

Analysis using GPower (GPower Version 3.1.9.2) showed that we could detect an association of moderate effect size (f^2=0.15) in our models with a power of 80%. We used Levene’s test to evaluate homogeneity of variances. Descriptive statistics are shown in mean ± standard deviation (continuous variables) or percentages (categorical variables). Linear regression analysis was performed to evaluate the main effect of HOMA-IR on the volumes of hippocampal subfields, adjusted by sex and the total brain volume. For the behavioral outcomes, linear regression analysis to evaluate the main effect of HOMA-IR on food preference and feeding behavior were adjusted by sex. When involving memory outcomes between the two time points, ANOVAs were adjusted by sex, age, time between visits and verbal fluency (comparison between participants who chose vs. did not choose their own snack were compared on insulin, HOMA-IR, glucose and BMI z scores).

In order to compare baseline characteristics and metabolic outcomes between groups according to birth weight (SGA or AGA), we used Pearson Chi-squared and Student’s T tests. Linear regression analysis to evaluate the main effect of birth status (SGA or AGA) on food preference and feeding behavior were adjusted by sex, and also the interaction between birth weight status and HOMA-IR on the same outcomes, followed by post-hoc simple slope analysis when indicated. Statistical significance was set at p<0.05.

3. Results

3.1 Sample characteristics

The sample was composed by 21 males and 31 females (59.6% females), 63.5% Caucasian ancestry, at a mean age of 17±2 years, mean IQ of 123.0±24.7 (WASI total) and phonemic and semantic verbal fluency of 42.3±10.7 (number of valid words NEUPSILIN-INF). Participants were mostly from socioeconomic classes B and C (medium SES, 65.4% of the participants). The mean time between the two visits was 6.6±2.4 months. Fifty-one subjects completed the entire snack choice test, while one participant did not answer the question about the snack’s spatial arrangement.

3.2 Structural and functional MRI

Table 1 describes the main effects of HOMA-IR on hippocampal total and subfields volumes. There was no statistically significant association between HOMA-IR and total Mucellini AB, Miguel PM, Dalle Molle R, Rodrigues DM, Machado TD, Reis RS, Toazza R, Salum GA, Bortoluzzi A, Franco AR, Buchweitz A, Barth B, Agranonik M, Nassim M, Meaney MJ, Manfro GG, Silveira PP. Diminished insulin sensitivity is associated with altered brain activation to food cues and with risk for obesity - Implications for individuals born small for gestational age. Appetite. 2022 Feb 1;169:105799. doi: 10.1016/j.appet.2021.105799
Table 2 demonstrates that there were no statistically significant main effects of HOMA-IR on feeding behavior (DEBQ), total calories, percentage of calories derived from carbohydrates, fats and proteins of the snack they chose at the research centre’s cafeteria. However, higher HOMA-IR values were significantly associated with lower snack caloric density (Table 2). Increased insulinemia [F(1,50)=4.193, p=0.046] and increased HOMA-IR index [F(1,50)=4.405, p=0.041] were seen in participants who did not choose their first original snack as compared to those who chose their own snack (Figure 2). The two groups did not differ in other variables such as z score BMI [chose own snack 0.29±1.15 kg/m2, chose other snack 0.86±1.17, F(1,50)=1.407, p=0.242] or glycemia [chose own snack 83.06±6.66 mg/dl, chose other snack 84.87±7.61, F(1,50)=1.133, p=0.293].

3.2 Food choices and feeding behavior

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3.3 Associations with birth status (main effects of SGA/AGA and interactions with HOMA-IR)

From the 52 subjects, 34 (65.4%) were classified as AGA, 15 (28.8%) were classified as SGA and 3 (5.8%) had no birth weight data available. The mean gestational age was similar between AGA and SGA adolescents (AGA 38.3±2.6 weeks gestation, SGA 39.6±1.2 weeks gestation, Student’s T Test p=0.07). There were no statistically significant differences regarding baseline characteristics such as sex, age, verbal fluency, intelligence quotient, ethnicity, maternal education or socioeconomic status between AGA and SGA groups (Supplemental Table 2). They also did not differ in terms of BMI, BMI z-score, glucose, insulin or HOMA-IR index levels. The only difference observed between the groups was related to the weight at birth, as expected (Supplemental Table 2).

Table 3 demonstrates that there were no statistically significant main effects of birth weight status on feeding behavior (DEBQ), total calories, percentage of calories derived from carbohydrates, fats and proteins of the snack they chose at the research centre’s cafeteria. However, being born SGA was significantly associated with higher snack caloric density (Table 3). Participants classified as SGA chose with less frequency their own snack based on the snack’s spatial arrangement in comparison to participants classified as AGA (Own snack based on special arrangement, SGA = 40%, AGA =80%, χ2=1.051, p=0.01). There were no differences in choice based on food composition (Own snack SGA = 80%, AGA =64.7%, Muccellini AB, Miguel PM, Dalle Molle R, Rodrigues DM, Machado TD, Reis RS, Toazza R, Salum GA, Bortoluzzi A, Franco AR, Buchweitz A, Barth B, Agranonik M, Nassim M, Meaney MJ, Manfro GG, Silveira PP. Diminished insulin sensitivity is associated with altered brain activation to food cues and with risk for obesity - Implications for individuals born small for gestational age. Appetite. 2022 Feb 1;169:105799. doi: 10.1016/j.appet.2021.105799.
χ^2=1.146, p=0.336). Moreover, there is a statistically significant interaction effect between the HOMA-IR index and the BWR on external eating behavior, in which in SGA individuals, a higher HOMA-IR is associated with increased external eating (although the simple slope fails to reach significance, B=0.469, p=0.07). There is no association between HOMA-IR and external eating in AGA individuals (simple slope B=-0.293, p=0.09).

4. Discussion

In this study we found that (1) diminished insulin sensitivity was correlated with activation in areas of visual attention and inactivation in areas associated with inhibitory control in healthy adolescents, when presented with images of hyperpalatable food contrasted with non-food objects. Insulin sensitivity was also associated with less consistency regarding implicit memory related to a consumed meal. Furthermore, (2) subjects who were born SGA showed implicit food memory inconsistency, and their external eating behavior was directly related to their insulin sensitivity.

When presented with images of palatable food contrasted with non-food objects, a higher insulin resistance index associated with lower palatable food activation in brain regions that influence inhibitory control, stimulus-driven attentional control and self-regulation [84-86]. Furthermore, as the insulin resistance index increased, there was higher activation in the cuneus, an area implicated in visual processing, valuation, and that influences the saliency of cues during decision-making [87]. This suggests that subjects who exhibited increased HOMA-IR index scores were more susceptible to tempting food cues [86] and they were more likely to identify palatable food cues as visually salient and rewarding [88]. When facing palatable food compared to healthy food images, the participants with higher insulin resistance index scores showed lower activation in sensorimotor areas related to the saliency of food cues and eating behavior [87], and they also demonstrated higher activation in the cuneus, reinforcing the hypothesis that subjects with increased HOMA-IR index scores had altered value and saliency to food. We also observed that HOMA-IR was associated with the volume of right CA4 and DG, and hippocampal subregion particularly sensitive to the damaging effects of early life adversity [89]. The results were very similar when using z-BMI or residualized HOMA-IR, suggesting that it is difficult to disentangle the effects of insulin resistance from obesity, at least at this young age.

Interestingly, individuals who did not choose their own snack had increased insulinemia and decreased insulin sensitivity. This behavioral outcome is in agreement with the imaging data, as poor insulin sensitivity associated with altered brain activation when presented with images of palatable food and there was modified attentional control to food cues. The altered implicit learned food preference in participants with decreased insulin sensitivity is interpreted as these individuals showing impaired food habituation, which is a memory phenomenon, that may be involved in the development of obesity [90, 91]. Altered stability in feeding patterns is associated with poor food choices, increased energy intake and greater weight gain [92, 93], probably because food choices are influenced by external cues, without attention to the individual’s current state of satiety or hunger. Therefore, implicit food memory impairment seems to be an early behavioral sign associated with changes in insulin sensitivity and, most importantly, precedes obesity [94]. Impaired implicit food memory may contribute to increased food intake [2, 95], leading to overeating, obesity and higher insulin resistance in the long-term, supporting the “vicious cycle” model proposed by Mucellini AB, Miguel PM, Dalle Molle R, Rodrigues DM, Machado TD, Reis RS, Toazza R, Salum GA, Bortoluzzi A, Franco AR, Buchweitz A, Barth B, Agranonik M, Nassim M, Meaney MJ, Manfro GG, Silveira PP. Diminished insulin sensitivity is associated with altered brain activation to food cues and with risk for obesity - Implications for individuals born small for gestational age. Appetite. 2022 Feb 1;169:105799. doi: 10.1016/j.appet.2021.105799

Martin & Davidson [96]. Our findings are also in agreement to studies showing that deficits in episodic memory are related to uncontrolled eating [97]. It has been shown that females with obesity have significantly poorer performance on source memory for both food and non-food cues than females with healthy weight [98], which is aligned to the suboptimal food-related decision theoretical model and to our results.

Anthropometric and biochemical measures in participants classified as SGA were not significantly different from participants classified as AGA, and this is likely due to the young age of the participants involved. We did find though that participants classified as SGA chose a snack with a higher caloric density than participants classified as AGA, suggesting that SGA subjects are at greater risk to develop obesity over time. A higher HOMA index score in SGA participants was also associated with increased external eating behavior. Individuals with a high external eating trait have an increased sensitivity to external reward cues and they have a reduced capacity to regulate their cognitive responses to food, showing a tendency to exhibit impulsive behavior when exposed to food-related motivational stimuli [99]. These results are in agreement with human studies and animal models that showed that food restricted subjects consume more food and are more impulsive eaters especially when presented with high-carbohydrate or high-fat foods [29, 30, 32, 34, 35, 38, 39, 77, 100, 101]. Insulin has traditionally been considered an important signaling molecule in regulating energy homeostasis and feeding behavior. Impaired insulin sensitivity associates with overeating and impairments in regulating food intake. Interestingly, binge eating behavior, besides being caused by psychological reasons is also influenced by increases and decreases in blood glucose levels following the consumption of carbohydrate rich foods [102]. Our findings are in accordance with the “thrifty phenotype” hypothesis wherein low birth weight is associated with long-term insulin resistance and this phenomenon is adaptive as long as food supplies remain scarce. In an environment where food supplies are abundant though, insulin resistance becomes a risk factor for the development of metabolic syndrome [27, 38].

Furthermore, individuals born small for gestational age were more likely not to choose the same snack consumed months earlier when the spatial arrangement was modified. This finding implies that these SGA individuals had an impaired memory related to a visual food cue and this may be related to altered eating behavior. Food variety, including variety in visual cues, affects palatability and energy intake [103-106] and not having a pattern in food plating may be one of the reasons for the increased consumption of food observed in individuals born SGA. The relevance of food arrangement has been previously demonstrated, indicating that individuals "eat first with their eyes", and they are willing to pay more for certain foods, to increase their liking for certain foods, to increase their intent to eat more of these foods when these foods are presented in a visually more appealing manner [107-109]. Meal layout planning also helps to decide on the kind and quantity of food to be eaten and is an adequate dietary intervention to reduce metabolic risk factors, including the glycemic index [110-112]. Besides that, implicit learned food preferences can contribute to susceptibility to metabolic syndrome if valenced attitudes toward foods are developed [71]. Displaying healthy foods in a more varied and attractive way or helping in the formation of a more rigid pattern of plating using implicit valenced cues, especially for palatable foods, The hippocampus is suggested to be a discriminatory retention brain region for food cues and memories related to eating [5, 113]. Furthermore, the inhibitory control of food intake and appetitive behavior depends on the functional integrity and structure of the hippocampus, thus this region is associated with what, when and how much to eat [114, Mucellini AB, Miguel PM, Dalle Molle R, Rodrigues DM, Machado TD, Reis RS, Toazza R, Salum GA, Bortoluzzi A, Franco AR, Buchweitz A, Barth B, Agranonik M, Nassim M, Meaney MJ, Manfro GG, Silveira PP. Diminished insulin sensitivity is associated with altered brain activation to food cues and with risk for obesity - Implications for individuals born small for gestational age. Appetite. 2022 Feb 1;169:105799. doi: 10.1016/j.appet.2021.105799
Considering that being born SGA persistently modifies insulin sensitivity from birth [20, 27] the relationships between HOMA-IR and hippocampal function and structure reported here may explain the altered feeding habits, impaired food memory (no plating pattern) and poor inhibitory control (higher intake and external eating related to insulin resistance) found in SGA individuals compared to individuals born AGA.

Insulin modulates feeding behavior through alterations in different brain circuitries. Insulin sensitivity correlates with the activity of dopaminergic neurons [116] and modulates feeding preferences in mesocorticolimbic pathways [117]. Insulin signaling also has been found to regulate dopamine neurotransmission, working together to orchestrate both the motivation to engage in consummatory behavior and to calibrate the associated level of reward [118], and these mechanisms could be playing a role in the described findings. Moreover, the hippocampus is involved in explicit food-related memory acquisition and recall, in the internal perception of satiety, in estimating time and meal length, in cued-food associations learning and in the control of stress-related eating [4], which are potential effects modified by insulin function in this structure. The difference in time (6 months) between lab visits [119, 120] and the type of food presentation (actual food versus photos of food items) [121] between the two lab visits should be taken into account when interpreting these results.

Our main limitation is the small sample size, which may affect our ability to detect differences between the groups. Future investigations using a larger sample size and other neuro-endocrine and eating related behavioral measures are necessary to further support our findings. The use of HOMA-IR as a marker of insulin sensitivity has also some limitations, as an OGTT-derived index of insulin sensitivity or an euglycemic hyperinsulinemic clamp would have been more robust measurements [122]. One of the strengths of this study was our ability to introduce a behavioral measure that could distinguish between individual variations in the HOMA-IR index and insulinemia. Previous behavioral studies about eating investigated hindbrain and midbrain circuits involved in the homeostatic and hedonic control of food intake [123, 124]. Our study demonstrated an altered mechanism that controls a cognitively related aspect of food intake, that is poorly explored in adolescents and had not been previously studied in individuals born SGA.

In conclusion, our work has contributed to highlight a mechanism involved in risky feeding behavior that could be associated with the development of obesity and metabolic syndrome. This mechanism is associated with insulin sensitivity and implicit food memory. Our findings also suggest a possible role for insulin sensitivity and the hippocampus in modulating feeding behavior in individuals born small for gestational age. Considering the significant incidence rates of intrauterine growth restriction found across many countries, furthering our understanding of the altered neurobiology associated with being born small for gestational age will help in the development of intervention and management programs that can improve risky eating behavioral patterns in this vulnerable population.

5. Acknowledgements

We thank the participants for their time and support. The authors report no conflicts of interest related to this work. The current study was supported by the National Council for Technological and Scientific Development (CNPq) (PPS, 478820/2010), Research and Event Incentive Fund of Hospital de Clínicas de Porto Alegre (FIPE/HCPA) (12-0254), Foundation for the Coordination of Higher Education and Graduate Training (CAPES), Canadian
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ABM, GGM and PPS were responsible for the study concept and design. ABM, RDM, DMR, TDM, RSR, RT, GAS and AB contributed to the acquisition of clinical data. ARF, AB, PMM, BB and MN assisted with MRI data acquisition and/or analysis. MA contributed to the statistical analysis of the study. ABM and PMM drafted the manuscript and GGM, PPS and MJM provided critical revisions on the manuscript for important intellectual content. All authors critically reviewed and approved the final version of the manuscript.


**Table 1.** Association between HOMA-IR and the volume of the hippocampus and its subfields (mm³).

<table>
<thead>
<tr>
<th>Brain structure</th>
<th>Main effect HOMA-IR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
</tr>
<tr>
<td>Right hippocampus</td>
<td>0.172</td>
<td>0.203</td>
</tr>
<tr>
<td>Left hippocampus</td>
<td>0.115</td>
<td>0.402</td>
</tr>
<tr>
<td>Right CA4 and DG</td>
<td>0.301</td>
<td>0.028</td>
</tr>
<tr>
<td>Left CA4 and DG</td>
<td>0.232</td>
<td>0.075</td>
</tr>
<tr>
<td>Right CA2 and CA3</td>
<td>0.293</td>
<td>0.053</td>
</tr>
<tr>
<td>Left CA2 and CA3</td>
<td>0.302</td>
<td>0.064</td>
</tr>
<tr>
<td>Right CA1</td>
<td>0.110</td>
<td>0.453</td>
</tr>
<tr>
<td>Left CA1</td>
<td>-0.014</td>
<td>0.928</td>
</tr>
<tr>
<td>Right str. rad. l-m.</td>
<td>0.069</td>
<td>0.639</td>
</tr>
<tr>
<td>Left str. rad. l-m.</td>
<td>0.051</td>
<td>0.734</td>
</tr>
<tr>
<td>Right subiculum</td>
<td>0.065</td>
<td>0.587</td>
</tr>
<tr>
<td>Left subiculum</td>
<td>0.045</td>
<td>0.713</td>
</tr>
</tbody>
</table>

Linear regression adjusted for total brain volume and sex; DG: dentate gyrus; Str. rad. l-m: stratum radiatum, lacunosum and moleculare.
**Table 2.** Association between HOMA-IR and feeding behavior (Dutch Eating Behavior Questionnaire) and analysis of the food choices during the Snack Test at the cafeteria.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Main effect HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
</tr>
<tr>
<td>Total Energy snack (kcal)</td>
<td>0.065</td>
</tr>
<tr>
<td>Energy from protein snack (%kcal)</td>
<td>-0.076</td>
</tr>
<tr>
<td>Energy from carbohydrate snack (%kcal)</td>
<td>-0.080</td>
</tr>
<tr>
<td>Energy from fat snack (%kcal)</td>
<td>0.165</td>
</tr>
<tr>
<td>Caloric density snack (kcal/g)</td>
<td>-0.310</td>
</tr>
<tr>
<td>DEBQ restrictive eating (score points)</td>
<td>0.243</td>
</tr>
<tr>
<td>DEBQ emotional eating (score points)</td>
<td>-0.183</td>
</tr>
<tr>
<td>DEBQ external eating (score points)</td>
<td>-0.080</td>
</tr>
</tbody>
</table>

Linear regression adjusted for sex.
Table 3. Association between birth status (SGA/AGA) main effect as well as interaction with HOMA-IR on feeding behavior (Dutch Eating Behavior Questionnaire) and analysis of the food choices during the Snack Test at the cafeteria.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Main effect of birth status (SGA=0, AGA=1)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>95% CI</td>
<td></td>
</tr>
<tr>
<td>Total Energy snack (kcal)</td>
<td>0.132</td>
<td>0.371</td>
<td>(-76.012; 199.765)</td>
<td></td>
</tr>
<tr>
<td>Energy from protein snack (%kcal)</td>
<td>0.146</td>
<td>0.321</td>
<td>(-1.522; 4.545)</td>
<td></td>
</tr>
<tr>
<td>Energy from carbohydrate snack (%kcal)</td>
<td>-0.178</td>
<td>0.222</td>
<td>(-11.236; 2.680)</td>
<td></td>
</tr>
<tr>
<td>Energy from fat snack (%kcal)</td>
<td>0.156</td>
<td>0.284</td>
<td>(-2.442; 8.063)</td>
<td></td>
</tr>
<tr>
<td>Caloric density snack (kcal/g)</td>
<td>-0.303</td>
<td>0.036</td>
<td>(-0.538; -0.019)</td>
<td></td>
</tr>
<tr>
<td>DEBQ restrictive eating (score points)</td>
<td>-0.170</td>
<td>0.231</td>
<td>(-10.621; 2.630)</td>
<td></td>
</tr>
<tr>
<td>DEBQ emotional eating (score points)</td>
<td>0.031</td>
<td>0.830</td>
<td>(-6.675; 8.280)</td>
<td></td>
</tr>
<tr>
<td>DEBQ external eating (score points)</td>
<td>-0.133</td>
<td>0.367</td>
<td>(-5.753; 2.166)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interaction birth status * HOMA-IR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>95% CI</td>
<td></td>
</tr>
<tr>
<td>Total Energy snack (kcal)</td>
<td>-0.167</td>
<td>0.730</td>
<td>(-131.931; 93.143)</td>
<td></td>
</tr>
<tr>
<td>Energy from protein snack (%kcal)</td>
<td>-0.152</td>
<td>0.753</td>
<td>(-2.875; 2.093)</td>
<td></td>
</tr>
<tr>
<td>Energy from carbohydrate snack (%kcal)</td>
<td>-0.434</td>
<td>0.361</td>
<td>(-8.228; 3.057)</td>
<td></td>
</tr>
<tr>
<td>Energy from fat snack (%kcal)</td>
<td>0.623</td>
<td>0.183</td>
<td>(-1.364; 6.952)</td>
<td></td>
</tr>
<tr>
<td>Caloric density snack (kcal/g)</td>
<td>-0.029</td>
<td>0.946</td>
<td>(-0.200; 0.187)</td>
<td></td>
</tr>
<tr>
<td>DEBQ restrictive eating (score points)</td>
<td>0.108</td>
<td>0.811</td>
<td>(-4.623; 5.876)</td>
<td></td>
</tr>
<tr>
<td>DEBQ emotional eating (score points)</td>
<td>-0.056</td>
<td>0.903</td>
<td>(-6.390; 5.660)</td>
<td></td>
</tr>
<tr>
<td>DEBQ external eating (score points)</td>
<td>-1.062</td>
<td>0.023</td>
<td>(-6.610; -0.507)</td>
<td></td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Research center’s cafeteria. Individuals received an equal sum of money to choose and buy a snack (A). The snack was photographed and in the subsequent visit, participants were asked which snack they would choose at that moment. Choices for both composition (B) as well as spatial arrangement (C) were investigated. In both cases, one of the pictures was of the original snack chosen and eaten by the participant.

Figure 2. Main results in the whole sample. (A) Positive correlation between the HOMA-IR index and right cuneus activation when presented with the contrast “images of palatable versus healthy foods” (SPM multiple regression analyses using HOMA-IR as regressor, \(P_{\text{FWR corr}}<0.001\)); (B) Negative correlation between the HOMA-IR index and left middle frontal gyrus activation when presented with the contrast “images of palatable foods versus non-food objects” (\(P_{\text{FDR corr}}=0.001\)); (C) Participants who chose another snack had an increased HOMA-IR index (ANOVA, \(p=0.044\)) and (D) Plasma insulin (\(p=0.045\)) when compared to those individuals who chose their own snack.