Manuscript Details

Manuscript number	APPETITE_2017_807_R1
Title	Fetal growth interacts with multilocus genetic score reflecting dopamine signaling capacity to predict spontaneous sugar intake in children
Article type	Full Length Article

Abstract

Background: We have shown that intrauterine growth restriction (IUGR) leads to increased preference for palatable foods at different ages in both humans and rodents. In IUGR rodents, altered striatal dopamine signaling associates with a preference for palatable foods. Objectives: Our aim was to investigate if a multilocus genetic score reflecting dopamine-signaling capacity is differently associated with spontaneous palatable food intake in children according to the fetal growth status. Methods: 192 four-year old children from a community sample from Montreal and Hamilton, Canada, were classified according to birth weight and administered a snack test meal containing regular as well as palatable foods. Intrauterine growth restriction was based on the birth weight ratio below 0.85; children were genotyped for polymorphisms associated with dopamine (DA) signaling, with the hypofunctional variants (TaqIA-A1 allele, DRD2-141C Ins/Ins, DRD4 7-repeat, DAT1-10-repeat, Met/Met-COMT) receiving the lowest scores, and a composite score was calculated reflecting the total number of the five genotypes. Macronutrient intake during the Snack Test was the outcome. Results: Adjusting for z-score BMI at 48 months and sex, there was a significant interaction of the genetic profile and fetal growth on sugar intake [= 4.56, p = 0.04], showing a positive association between the genetic score and sugar intake in IUGR children, and no association in non-IUGR children. No significant interactions were seen in other macronutrients. Conclusions: Variations in a genetic score reflecting DA signaling are associated with differences in sugar intake only in IUGR children, suggesting that DA function is involved in this behavioral feature in these children. This may have important implications for obesity prevention in this population.

Keywords	IUGR, dopamine, multilocus score, palatable food intake
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Suggested reviewers	Antonio Gonzalez-Bulnes, Michael Michaelides

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Montreal, October 2nd, 2017.

Dr. Nori Geary
Editor
Appetite

Dear Dr. Geary:

Please find attached the reviewed manuscript entitled "**Fetal growth interacts with multilocus genetic score reflecting dopamine signaling capacity to predict spontaneous sugar intake in children**" as well as the point by point reply to the Reviewer's comments for consideration.

Thank you very much for the interesting suggestions that certainly improved our work. We hope that you will find the responses and the modifications acceptable.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,

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Reply to Reviewers Comments
Editor:
Please use "sex" rather than "gender" in accordance with Appetite's Guidelines.
This was corrected throughout the manuscript as requested.

Reviewer 1:

General
This is an interesting paper. I believe that the correlations may be sufficiently interesting to merit publication, but that the authors do not highlight the weakness of the data strongly enough.
Thank you for the comments and we hope that the corrected version is more suitable.

Major
The main weakness as I understand it is that the key result is not really established. The authors write, "Post hoc analysis showed that in the IUGR group there is an increased intake of sugars [estimated β=4.01, p=0.04] as a function of an increased multi-locus, high DA signaling genotype score." They discuss this as though it is specific to the IUGR children. I believe that the key point is whether the DA gene score – intake differs in IUGR and non-IUGR children. It is a basic tenet of statistical hypothesis testing that one significant result (sentence above) and one non-significant result (non-IUGR group) does not prove that the two are different. If this cannot be shown, then it needs to be discussed as a limitation and the data need to be described as merely suggestive.
In fact, our main finding shows that the effect of the multilocus score on sugar intake is different between IUGR and non-IUGR children, which is demonstrated by the significant interaction coefficient [$\hat{\beta}$ =-4.56, p=0.042]. The analysis of the simple slopes shows that in IUGR children there was a positive association between the genetic score and sugar intake [$\hat{\beta}$ =4.01, p=0.04]. No association between the multilocus score and sugar intake was seen in non-IUGR children [$\hat{\beta}$ =-0.54, p=0.60].

According to the suggestions, we edited the description of the Results to make them clearer:

"As seen in Table 3, a linear regression analysis showed a significant interaction between IUGR status and the multilocus score for sugars intake [$\hat{\beta}$ =–4.56, p=0.04] during the Snack Test. The effect was specific for sugars as there were no significant interactions observed between IUGR status and the multilocus score for complex carbohydrates [$\hat{\beta}$ =–2.62, p=0.20], fiber [$\hat{\beta}$ =–0.39, p=0.19], protein [$\hat{\beta}$ =–0.72, p=0.55] or fat [$\hat{\beta}$ =–0.37, p=0.82]. Simple slopes analysis showed that in the IUGR group there was a positive relationship between the multilocus score (DA signaling genotype score) and the intake of sugars [$\hat{\beta}$ =4.01, p=0.04]. In other words, variations in dopamine signaling capacity were associated with differences in the consumption of sugars in IUGR children only. There was no significant effect of the dopamine multilocus genetic score on sugars intake in non-IUGR children [$\hat{\beta}$ = –0.54, p=0.60], Figure 1 A."

Other

(line) 6 – Does the first sentence refer to humans or rodents?

We and others have described increased palatable foods in IUGR individuals both in humans (Barbieri et al., 2009, Ayres et al., 2012, Lussana et al., 2008, Migraine et al., 2013) and rodents (Alves et al., 2015, Dalle Molle et al., 2015, Laureano et al., 2016). This was clarified in the abstract.

35 – Please describe results in past tense throughout.
This was corrected as requested.

102 – No need for a double conditional. Suggest "A potential mechanism linking prenatal growth with feeding behavior and postnatal metabolic risk is changes in the central nervous system circuitry that underlie palatable food intake."
Thank you, this was corrected.

100 – Why not just "eating" (feeding is a behavior, so "feeding behavior" is redundant, and "feed" can mean either "provide food for" or "eat food", whereas "eat" is just the latter. I recommend this change throughout.
This was corrected as requested.

112 – Incentive rewards is only a single reward mechanism, is variously defined, and is not crucial to this work. I suggest simply "reward" or "hedonics" here. "Metabolic status" is completely unclear here. I would delete it. Perhaps, "…integrates information related to both hedonic and homeostatic food stimuli."
This was corrected as requested.

136 – The last two sentences should be merged as they mean the same thing. The second adds only the term "moderate."
This was corrected as requested.

232 – "Exposed to" should be "offered." What were the instructions?
This was corrected. As mentioned in the manuscript, mothers were asked to offer a light breakfast to participants at home beforehand (milk/bread but not eggs or bacon) and literally asked not to share plates or encourage/inhibit the intake of specific foods to avoid influencing the children's choices.

234 – Please give the manufacturers for any processed foods used.
250 – "would have" should be "had."
254 "orientation" should be "assistance."
These were corrected as requested.

260 – I do not understand "share plates." Do you mean not share food chosen by the child?
Mother and child had each a set of plates with the different types of foods on it, and they were not supposed to share.

273 – "Various efforts were made…" So the procedure was not standardized in some respects? Which? It would be better to identify specific aspects that varied during the course of the experiment. I assume this is a minor issue, but would like more details.

Understandably, there were minor variations between the subjects, e.g. the precise time of the test start (between 10-11h), some children did not need a cushion, etc. What we mean by this sentence is that despite these small inevitable variations we indeed were careful to make this experiment as most "natural" and "standard" possible for a 4-year-old child eating a Snack in the lab, as well as to register any form of deviance on the charts.

290 – "The genetic model was driven by the biological function." I have no idea what this means; please clarify.
The 5 polymorphisms that compose the genetic score have well defined biological function described by molecular studies. In other words, we know the molecular effect of carrying that specific polymorphism (increase or decrease dopamine biological function), and this defined the choice of these specific gene variants for composing the score. We edited the text to clarify this.

306 – "receipt" is too general. Do you mean sight of, consumption, or what?
It's consumption, and this was corrected as requested.

337 – should be "Student's t test" (throughout)
346 – suggest "IUGR was analyzed as a categorical variable (normal or IUGR)" rather than "Considering the clinical relevance of IUGR as a vulnerability factor, we opted for using this variable as categorical in the analysis and for graphing the results in Figure 1."
372-452- Please use only past tense to describe results. Do not alternate!
376 – omit "it"
These were corrected as requested.

385, 387 – Do you really mean "simple sugars"? I think this term is not used in a standard way. I understand simple sugars to mean monosaccharides, but I assume you mean mono- or disaccharides. And what are "simple carbohydrates"? Do you now mean to include oligosaccharides (as in high fructose corn syrup)?
In fact, simple sugars and simple carbohydrates were used as synonyms throughout the text. We understand that this may be confusing and therefore we corrected it as requested and left only "sugars", "complex carbohydrates" and "fiber".

434 - Should be "outlier" rather than "influential point." Is it really an outlier? If you consider all the intakes ignoring gene scores, is this point an outlier? (or the low value at gene score 3)?
The point mentioned by us is not the same that the reviewer is referring to. The observation that we mention belongs to the IUGR group, and has low genetic score and high intake of sugar (Please see Figure 1). This observation is indeed an outlier and an influential point. This was clarified in the Results section:

"(...)the graph suggests the existence of one outlier in the IUGR group with low multilocus score/high sugar intake. We performed a diagnostic check to see if this observation would have an impact on the regression coefficients, using a measure of influence, DFBETAS, which characterized it as an influential point. When excluding this subject from the analysis, the interaction between IUGR status and the genetic score became significant for total caloric consumption ($\hat{\beta}$=–58.38, p= 0.04), and remained significant for sugars ($\hat{\beta}$ =–6.17, p<0.01), but

not for complex carbohydrates ($\hat{\beta}$=–3.70, p=0.08), fiber ($\hat{\beta}$=–0.49, p=0.11), protein ($\hat{\beta}$=–1.51, p=0.22), or fat ($\hat{\beta}$=–1.24, p=0.46).”

468 – “Additionally” rather than “accordingly”
543 – “with regard to” rather than “with regards to”
594 – Do not capitalize “Pediatric Care”
Table 2 – should be “p” not “P” to coordinate with rest of manuscript.
Table 4 – Data are reported in a totally unrealistic degree of precision. Please round to 1 kcal, g, % (and even that is probably more precise than truly the case).
These were corrected as requested.

Fig 1 – The crucial comparison is whether the two regression lines differ significantly, especially in slope. Do they? The figure is unclear due to the many points – I suggest breaking the IUGR and non-IUGR into separate scatter plots.
As explained above, yes, the slopes are significantly different. The figures were adjusted according to the request:
Fig 1A

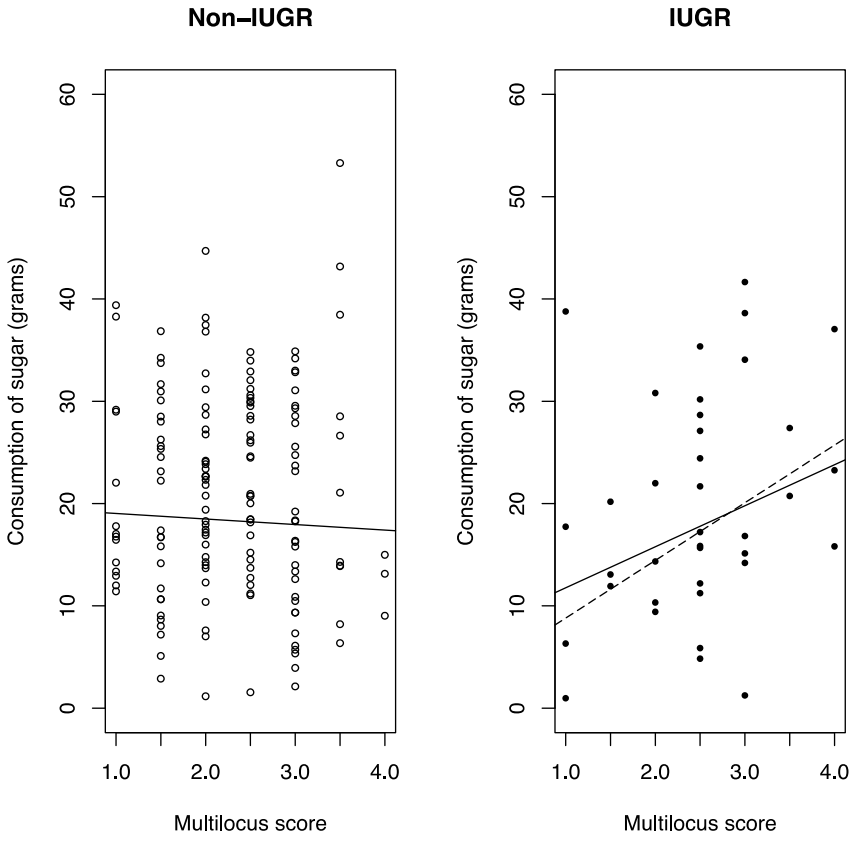
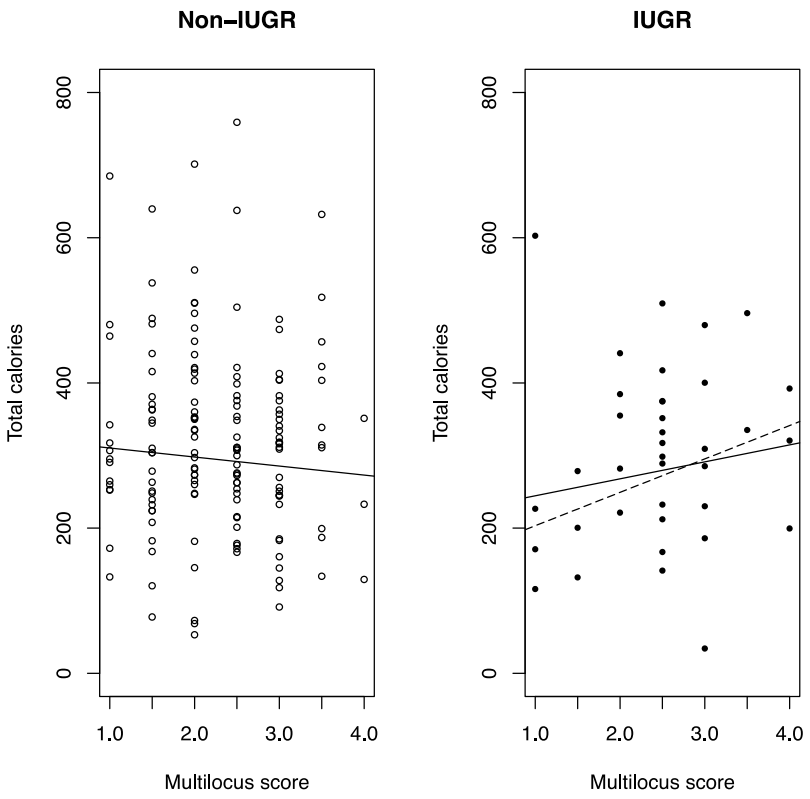


Fig 1 B



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ABSTRACT

Background: We have shown that intrauterine growth restriction (IUGR) leads to increased preference for palatable foods at different ages in both humans and rodents. In IUGR rodents, altered striatal dopamine signaling associates with a preference for palatable foods. **Objectives**: Our aim was to investigate if a multilocus genetic score reflecting dopamine-signaling capacity is differently associated with spontaneous palatable food intake in children according to the fetal growth status. **Methods**: 192 four-year old children from a community sample from Montreal and Hamilton, Canada, were classified according to birth weight and administered a snack test meal containing regular as well as palatable foods. Intrauterine growth restriction was based on the birth weight ratio below 0.85; children were genotyped for polymorphisms associated with dopamine (DA) signaling, with the hypofunctional variants (TaqIA-A1 allele, DRD2-141C Ins/Ins, DRD4 7-repeat, DAT1-10-repeat, Met/Met-COMT) receiving the lowest scores, and a composite score was calculated reflecting the total number of the five genotypes. Macronutrient intake during the Snack Test was the outcome. **Results:** Adjusting for z-score BMI at 48 months and sex, there was a significant interaction of the genetic profile and fetal growth on sugar intake [$\hat{\beta}$ = –4.56, p = 0.04], showing a positive association between the genetic score and sugar intake in IUGR children, and no association in non-IUGR children. No significant interactions were seen in other macronutrients. **Conclusions**: Variations in a genetic score reflecting DA signaling are associated with differences in sugar intake only in IUGR children, suggesting that DA function is involved in this behavioral feature in these children. This may have important implications for obesity prevention in this population.

Fetal growth interacts with multilocus genetic score reflecting dopamine signaling capacity to predict spontaneous sugar intake in children

Running title: Dopamine and sugar intake in IUGR children

Keywords: IUGR, dopamine, multilocus score, palatable food intake

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ABSTRACT

Background: We have shown that intrauterine growth restriction (IUGR) leads to increased preference for palatable foods at different ages in both humans and rodents. In IUGR rodents, altered striatal dopamine signaling associates with a preference for palatable foods. **Objectives**: Our aim was to investigate if a multilocus genetic score reflecting dopamine-signaling capacity is differently associated with spontaneous palatable food intake in children according to the fetal growth status. **Methods**: 192 four-year old children from a community sample from Montreal and Hamilton, Canada, were classified according to birth weight and administered a snack test meal containing regular as well as palatable foods. Intrauterine growth restriction was based on the birth weight ratio below 0.85; children were genotyped for polymorphisms associated with dopamine (DA) signaling, with the hypofunctional variants (TaqIA-A1 allele, DRD2-141C Ins/Ins, DRD4 7-repeat, DAT1-10-repeat, Met/Met-COMT) receiving the lowest scores, and a composite score was calculated reflecting the total number of the five genotypes. Macronutrient intake during the Snack Test was the outcome. **Results:** Adjusting for z-score BMI at 48 months and sex, there was a significant interaction of the genetic profile and fetal growth on sugar intake [$\hat{\beta}$ = –4.56, p = 0.04], showing a positive association between the genetic score and sugar intake in IUGR children, and no association in non-IUGR children. No significant interactions were seen in other macronutrients. **Conclusions**: Variations in a genetic score reflecting DA signaling are associated with differences in sugar intake only in IUGR children, suggesting that DA function is involved in this behavioral feature in these children. This may have important implications for obesity prevention in this population.

INTRODUCTION

Intrauterine growth restriction (IUGR) refers to a situation in which the fetus does not reach its full growth potential during pregnancy (Chatelain, 2000). IUGR results from placental dysfunction, which occurs in many prevalent conditions during gestation such as infections, hypertension, drug and tobacco exposure, as well as under or over nutrition (Nohr, et al., 2005). The prevalence of IUGR is constant worldwide, affecting 7-15% of all births independent of regional economic development (Organization, 2004).

Epidemiological studies show that impaired fetal growth reflected in low birth weight is associated with increased risk for cardiovascular disease (Barker, Winter, Osmond, Margetts, & Simmonds, 1989; C. E. Stein, et al., 1996), type II diabetes (Hales & Barker, 1992; Phipps, et al., 1993), and increased adiposity (Bettiol, et al., 2007; Ravelli, Stein, & Susser, 1976) in adulthood. We and others provided evidence suggesting that IUGR individuals have altered food preferences from early infancy until adult age, favoring the intake of palatable foods that are rich in sugar and/or fat (Ayres, et al., 2012; Barbieri, et al., 2009; Lussana, et al., 2008). This "thrifty-eating" phenotype could contribute to the development of chronic metabolic dysfunction.

A potential mechanism linking prenatal growth with altered eating and postnatal metabolic risk is changes in the central nervous system circuitry that underlie palatable food intake. The over-consumption of palatable or 'rewarding' foods likely reflects an imbalance in the relative importance of hedonic versus homeostatic signals (Egecioglu, et al., 2011). Central to the neurobiology of the hedonic mechanisms is the mesolimbic dopamine (DA) system, which receives and integrates information related to both hedonic and homeostatic food stimuli (Murray, Tulloch, Gold, & Avena, 2014).

We have shown that dopamine-related behaviors such as impulsivity (Silveira, et al., 2012) and poor inhibitory control (Reis, et al., 2015; Reis RS, 2016) are important moderators of the association between IUGR and altered eating in children and adolescents. We also demonstrated that differential dopamine signaling in cortical and striatal regions is implicated in the specific adult food preferences associated with IUGR in rodents (Alves, Molle, Desai, Ross, & Silveira, 2015; Molle, et al., 2015). Based on these various findings, we hypothesized that the exposure to an adverse environment culminating in IUGR moderates the association between a multilocus genetic score reflecting dopamine functioning and the consumption of palatable foods (sugar and/or fat) in preschool children.

MATERIAL AND METHODS

General Method

We used data from an established prospective birth cohort (Maternal Adversity, Vulnerability and Neurodevelopment - MAVAN) (O'Donnell, 2014). The study sample included 4-year old children from Montreal (Quebec) and Hamilton (Ontario), Canada. Eligibility criteria for mothers included age ≥18 years old, singleton pregnancy, and fluency in French or English. Mothers were excluded from the study if they had severe chronic illness, placenta previa, a history of incompetent cervix, impending delivery, or had a fetus/infant born at gestational age <37 weeks or born with a major anomaly. Birth records were obtained directly from the birthing units. Dyads were assessed longitudinally, with multiple assessments of both mother and child in home and laboratory across the child's development. Approval for the MAVAN project was obtained from obstetricians performing deliveries at the study hospitals and by the institutional review boards at hospitals and university affiliates:

McGill University, l'Université de Montréal, the Royal Victoria Hospital, Jewish General Hospital, Centre Hospitalier de l'Université de Montréal, Hôpital Maisonneuve-Rosemont, St Joseph's Hospital, and McMaster University. Informed consent was obtained from the parents/guardians of the participants.

At 4 years of age children came to the laboratory for various food-related tasks, and their standing height, without shoes, was measured (to the nearest 0.1 cm) with the use of a stadiometer (Perspective Enterprises, PE-AIM-101, Portage, Michigan). Body weight, in light clothing, was measured (to the nearest 0.1 kg) with the use of a digital floor scale (TANITA BF625, Arlington Heights, Illinois). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). We calculated the z-scores for BMI at 48 months according to World Health Organization (WHO) standards (WHO, 2006).

Fetal growth restriction definition

The definition of IUGR was based on the birth weight ratio (BWR), namely, the ratio between the birth weight and the sex-specific mean birth weight for each gestational age for the local population (Kramer, et al., 2001). A BWR of <0.85 was classified as IUGR (Kramer, Platt, Yang, McNamara, & Usher, 1999).

Snack test

Children and mothers were offered a test meal at approximately 10:30 a.m. including different types of foods in pre-weighed portions for 30 min: Frosted Flakes® (Kellogg's), sliced apple, muffin with chocolate drops, 3.25% milk, maple syrup flavored baked beans, croissant, cooked egg, cheddar cheese, All Bran ® (Kellogg's), white bread, orange juice (Levitan, et al., 2015; Silveira, et al., 2014).

Pre-weighed plates of the different foods were displayed in a buffet to which the child had total access. At the end of the session, the remaining foods were weighed again to measure the intake. Foods were chosen with the assistance of a nutritionist to represent local habitual snack items and to have similar colours (Addessi, Galloway, Visalberghi, & Birch, 2005). Mothers were instructed to offer a light breakfast to participants at home beforehand and not to share plates or influence the children's choices. Based on the nutritional content of each food and the amount eaten, we calculated the amount of fat, carbohydrates and protein ingested (Vozzo, et al., 2003). The test meal was eaten in the laboratory, in a 30 m^2 room. A table with two sets of plates was placed in the center of the room, with chairs for mother and child on both sides (facing each other). A cushion was placed on the child's chair to facilitate accessibility of the different foods. Various efforts were made to standardize this procedure between subjects.

Genetic data and multilocus score definition

Saliva samples were collected and genotyping of the DNA was performed blind to the children's behavior and phenotype. The five polymorphisms, later used to create the multilocus genetic score, ANKK1/DRD2 markers (rs1800497 [Taq1A]), COMT Val158Met (rs4680) SNP, DRD2 rs1799732 [−141delC], DAT1 and DRD4 VNTRs were amplified with polymerase chain reaction (PCR) techniques with primers and conditions previously described(Davis, et al., 2013). The construction of the multilocus genetic score was based on the biological functions described in the literature and on the approach proposed by Stice et al(Stice, Yokum, Burger, Epstein, & Smolen, 2012), who showed that this multilocus genetic composite is positively correlated with the degree of activation of different brain regions in response to

milkshake intake in contrast to a tasteless solution receipt. In this score, genotypes associated with putatively low DA signaling received a score of 0; those associated with putatively high DA signaling received a score of 1; intermediate heterozygotes received a score of 0.5. Specifically, TaqIA A1/A1 (Noble, Blum, Ritchie, Montgomery, & Sheridan, 1991), DRD2-141C Ins/Ins carriers (Jonsson, et al., 1999), DRD4-7 repeat carriers (Asghari, et al., 1995), DAT1 10R/10R (Mill, Asherson, Browes, D'Souza, & Craig, 2002), and COMT Met/Met (Lachman, et al., 1996) genotypes were assigned a score of 0 ("low"); TaqIA A2/A2, DRD2-141C Del/Del carriers, DRD4 non 7-repeat carriers, DAT1 9/9 carriers, and COMT Val/Val genotypes were assigned a score of 1 ("high"), and DRD2-141C Ins/Del, TaqIA A1/A2, DAT 1 9/10 and COMT Met/Val genotypes received a score of 0.5. The scores were then summed to create a multilocus composite.

Statistical analysis

Statistical analysis of the baseline characteristics was performed using Student's t-test for continuous data and chi-square test for categorical variables. A series of linear regression models were performed to investigate the association between fetal growth and the multilocus score as independent variables on the intake of the different macronutrients during the Snack Test, adjusting for BMI at the time of the test and sex. IUGR was analyzed as a categorical variable (normal birth weight or IUGR). Additional analyses were performed adjusting for ethnicity (children classified as Caucasians and non-Caucasians). Data were analyzed using the Statistical Package for the Social Sciences (SPSS) 22.0 software (SPSS Inc., Chicago, IL, USA). Significance levels for all measures were set at $p< 0.05$.

RESULTS

One hundred and ninety-two 48-month-old children were classified as IUGR or normal birth weight. Children born IUGR or not did not differ in many confounders as can be seen in *Table 1*. Genotype distribution for each gene is depicted in *Table 2*. Hardy-Weinberg equilibrium criteria were met in all cases, except for DRD4 7-repeat (p=0.04).

As seen in Table 3, a linear regression analysis showed a significant interaction between IUGR status and the multilocus score for sugars intake [$\hat{\beta}$ =–4.56, p=0.04] during the Snack Test. The effect was specific for sugars as there were no significant interactions observed between IUGR status and the multilocus score for complex carbohydrates [$\hat{\beta}$ =–2.62, p=0.20], fiber [$\hat{\beta}$ =–0.39, p=0.19], protein [$\hat{\beta}$ =–0.72, p=0.55] or fat [$\hat{\beta}$ =–0.37, p=0.82]. Simple slopes analysis showed that in the IUGR group there was a positive relationship between the multilocus score (DA signaling genotype score) and the intake of sugars [$\hat{\beta}$ =4.01, p=0.04]. In other words, variations in dopamine signaling capacity were associated with differences in the consumption of sugars in IUGR children only. There was no significant effect of the dopamine multilocus genetic score on sugars intake in non-IUGR children [$\hat{\beta}$ = –0.54, p=0.60], Figure 1 A.

See figure 1.

Figure 1B depicts the data for total calories consumed during the Snack Test in IUGR and non-IUGR children. Although there were no significant interactions between IUGR and the multilocus genetic score on total caloric intake as seen in Table 3 [$\hat{\beta}$ =–35.82, p=0.21], the graph suggests the existence of one outlier in the IUGR group with low multilocus score/high sugar intake. We performed a diagnostic check to see if this observation would have an impact on the regression coefficients,

using a measure of influence, DFBETAS, which characterized it as an influential point. When excluding this subject from the analysis, the interaction between IUGR status and the genetic score became significant for total caloric consumption ($\hat{\beta}$ =–58.38, p= 0.04), and remained significant for sugars ($\hat{\beta}$ =–6.17, p<0.01), but not for complex carbohydrates ($\hat{\beta}$=–3.70, p=0.08), fiber ($\hat{\beta}$=–0.49, p=0.11), protein ($\hat{\beta}$ =–1.51, p=0.22), or fat ($\hat{\beta}$=–1.24, p=0.46).

We also repeated the analysis adjusting for ethnicity. The results were similar to the previously described: the interaction between IUGR and the genetic score remained significant for sugars ($\hat{\beta}$=–5.42, p=0.03), but not for total caloric consumption ($\hat{\beta}$=–32.58, p= 0.32), for complex carbohydrates ($\hat{\beta}$=–2.29, p=0.32), fiber ($\hat{\beta}$=–0.27, p=0.45), protein ($\hat{\beta}$=–0.22, p=0.88), or fat ($\hat{\beta}$=0.04, p=0.98).

IUGR and non-IUGR children did not differ in the consumption of the different macronutrients as shown in Table 4.

DISCUSSION

We showed here an interaction between fetal growth and a dopamine multilocus genetic score, suggesting that variation in the dopamine signaling capacity is positively correlated to spontaneous sugar intake in IUGR children at 48 months of age. This is in agreement to our study in rodents (Molle, et al., 2015), in which altered levels of accumbal D2 receptors accompanied the increased preference for palatable foods in IUGR rats. Additionally, positron emission tomography studies show that the availability of striatal dopamine D2 receptor is decreased in obese individuals (Wang, et al., 2001). Brain fMRI studies demonstrate that individuals with elevated multilocus composite scores show less activation in the striatum in response to monetary reward (Nikolova, Ferrell, Manuck, & Hariri, 2011; Stice, et al., 2012).

Moreover, a higher multilocus score is associated with food addiction, binge eating, food cravings and emotional overeating (Davis, et al., 2013).

IUGR persistently affects the functioning of neuroendocrine axis such as the hypothalamic-pituitary-adrenal (HPA) axis (Osterholm, Hostinar, & Gunnar, 2012), as well as the sensitivity to insulin (Hales & Barker, 1992) and leptin (Desai, Gayle, Han, & Ross, 2007), and these hormones are known modulators of the mesolimbic dopaminergic system (Murray, et al., 2014; Rouge-Pont, Deroche, Le Moal, & Piazza, 1998). For instance, variations in the HPA responsivity to acute stress influence palatable food intake in women (Epel, Lapidus, McEwen, & Brownell, 2001). Insulin sensitivity is inversely associated with activation in the anterior cingulate, insula, orbitofrontal cortex and the frontal and rolandic operculum (Adam, et al., 2015). It has been shown that while mild hypoglycemia activates limbic-striatal brain regions in response to food cues to produce a greater desire for high-calorie foods, euglycemia activates the medial prefrontal cortex and decreases interest in food stimuli (Page, et al., 2011). Therefore, it makes sense that variations in the sensitivity to glucocorticoids and insulin associated with fetal programming interact with the mesocorticolimbic response to palatable foods, consequently affecting intake as seen in the current study. In agreement with previous findings (Barbieri, et al., 2009), we show here once more that the link between low birth weight and increased palatable food intake occurs before obesity emerges, and therefore is not secondary to its consequent metabolic disarrangements; these subtle nutritional differences may in fact mediate the development of adiposity in IUGR individuals, as proposed before (Portella, et al., 2012; Portella & Silveira, 2014a; Silveira, et al., 2012).

With regard to the specificity to sugar, as mentioned above, it is in agreement to our previous data (Barbieri, et al., 2009). However, other studies have found

associations between low birth weight and other types of preference. For instance, studies of the Dutch famine have shown preference towards fats in older adults whose mothers were exposed to the famine (Lussana, et al., 2008; A. D. Stein, Rundle, Wada, Goldbohm, & Lumey, 2009). *Perälä et al* had similar findings, with a positive correlation of small size at birth and increased consumption of fats, as well as a lower intake of carbohydrates, sucrose, fructose, fiber and fruits in adults of 56 to 70 years old (Perälä, et al., 2012). In a different sample, *Kaseva et al* demonstrate a higher intake of polyunsaturated fatty acids and essential fatty acids, and reduced use of vegetables, fruits, and milk products in very low birth weight at 19-27 years of age (Kaseva, et al., 2013). It appears to us that despite the apparent discrepant food preferences described in the several studies, we should consider that these were performed in different ages and using diverse tools (questionnaires, food diaries and actual consumption, as in the current work). All of these variables may explain the differences (for instance, food preferences change as the individuals age)(Cooke & Wardle, 2005); in addition, all studies seem to converge to an increased intake of palatable foods (sugar and/or fats) in IUGR children (Portella & Silveira, 2014b).

This study reinforces the idea that IUGR associates with persistent alterations in the brain circuitry related to palatable food intake and energy expenditure (Alves, et al., 2015; Cunha Fda, et al., 2015; Molle, et al., 2015). The importance of the current findings resides on the early identification of vulnerability to increased adiposity and its metabolic consequences, prompting the proposal of preventive measures and careful consideration of food preferences in these children in early pediatric care.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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FIGURE AND TABLES LEGENDS

Table 1 - Study participants' baseline characteristics according to IUGR status. [a]Student's t-test and [b]chi-square test. Data are expressed as mean (standard deviation), or proportions (percentages). LICO=Low Income Cut Off. Differences between IUGR and non-IUGR groups were not significant for all variables shown (all p-values >0.05).

Table 2 – Genotype distribution in the study sample. Criteria for Hardy Weinberg Equilibrium were met for all genes except for DRD4 VNTR.

Table 3 – Estimated beta coefficients for analyses of different macronutrients. The baseline on the analysis was the IUGR group and female sex.

Table 4 - Consumption, displayed as means (standard deviations), of the different macronutrients by IUGR and non-IUGR children. No significant differences in consumption were seen between the groups.

Figure 1 – Association between the multilocus score and sugars (A) and calories (B) intake in IUGR and non-IUGR children. Variation in the genetic score is associated with different sugar intake only in IUGR children. Dotted line represents the regression line when excluding an influential observation (see text for details). The predicted values for sugar consumption and total calories consumption were shown for females and z-BMI at 48 months=0.54.

Table 1 - Description of the baseline characteristics of the sample

Sample characteristics	Non IUGR (n=155)	IUGR (n=37)	p-values
Females (%)[b]	50% (77)	51% (19)	0.85
Maternal age at birth (y) [a]	30.8 (4.6)	29.5 (4.9)	0.15
Maternal smoking during gestation (%)[b]	10% (14)	24% (7)	0.06
Maternal education below 10 years of schooling (%)[b]	3% (5)	6% (2)	0.62
Family income below LICO (%)[b]	16% (23)	17% (6)	0.95
Total duration of breastfeeding (weeks)[a]	29 (19)	28 (20)	0.74

Table 2. Genotype distribution in the study sample.

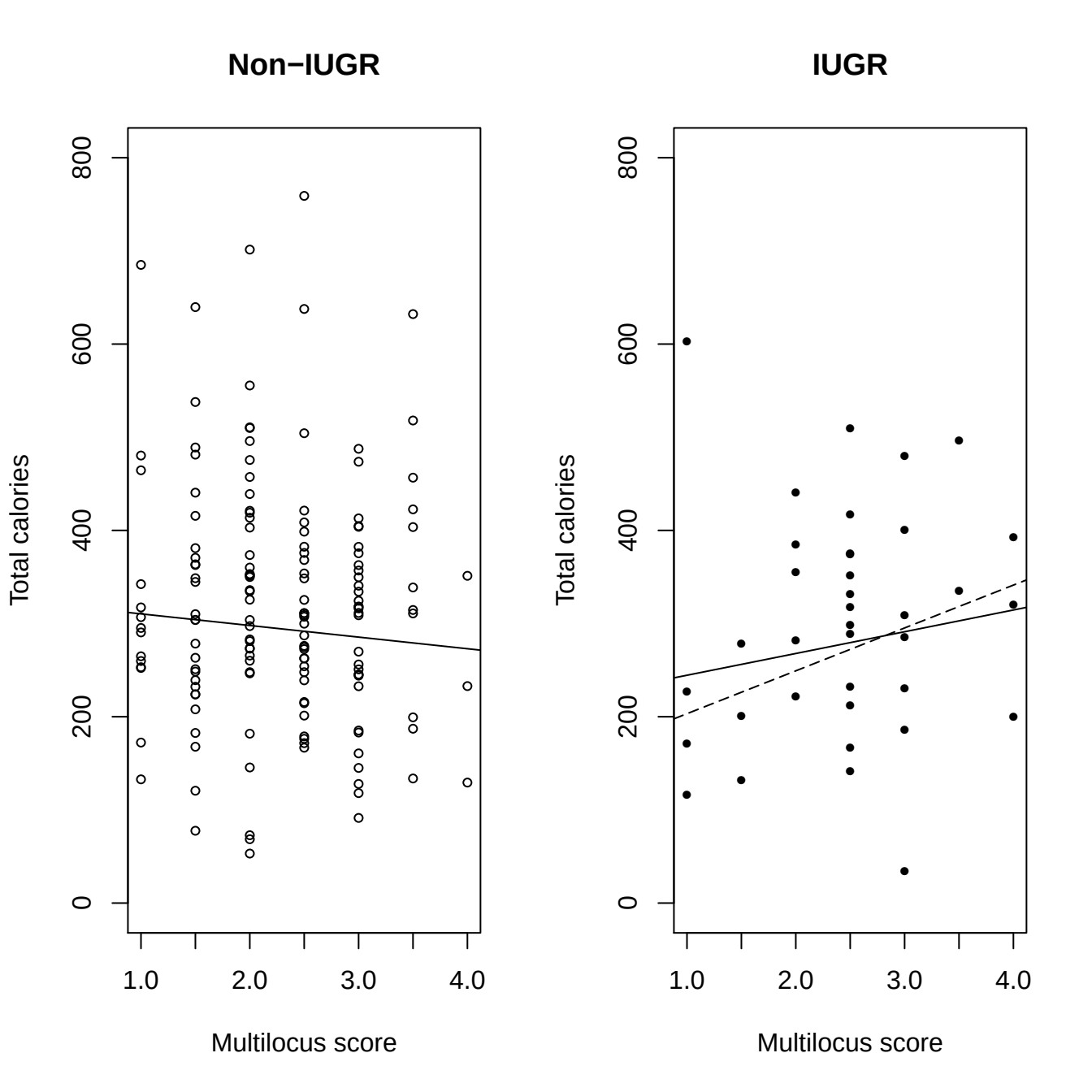
Gene		H-W equilibrium
DAT1 VNTR	10/10 (104, 54.2%); 9/10 (73, 38%); 9/9 (15, 7.8%)	p=0.66
DRD2 141C (rs1799732) BstNl	Ins/Ins (142, 74%); Ins/Del (46, 24%); Del/Del (4, 2.1%).	p=0.90
DRD4 VNTR	7R homozygous (4, 2.08%); 7R heterozygous (74, 38.5%); non-7R/non-7R (114, 59.4%)	p=0.04
Taq IA (rs1800497)	A1/A1 (10, 5.2%); A1/A2 (55, 28.6%); A2/A2 (127, 66.1%)	p=0.22
COMT (rs4680)	A/A (49, 25.5%); A/G (92, 47.9%); G/G (51, 26.6%)	p=0.56

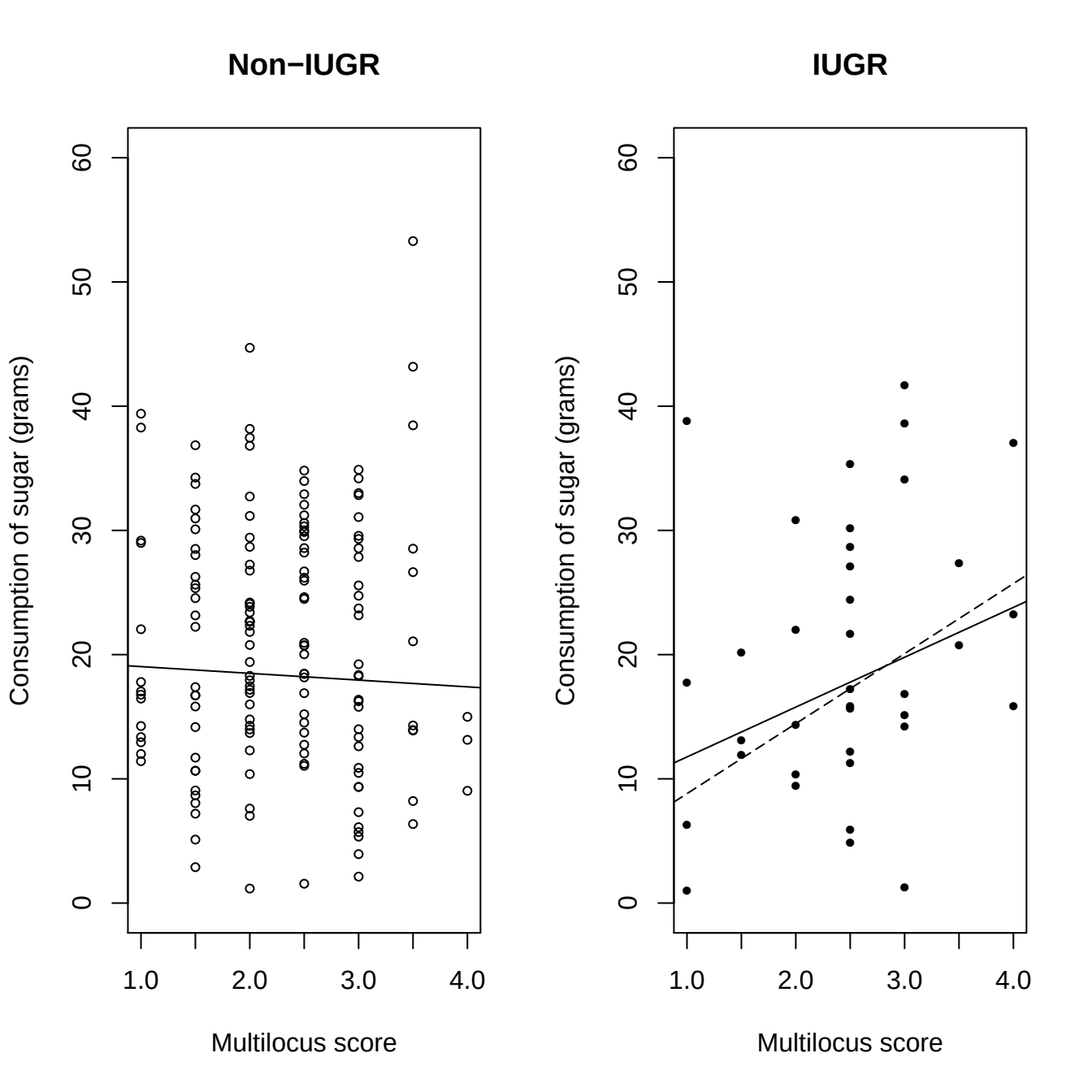
Table 3. Estimated beta coefficients for analyses of different macronutrients consumption

Macronutrients		$\hat{\beta}$	p-values
Total calories consumption	z-BMI 48m	25.60	<0.01
	IUGR status	101.69	0.16
	Sex	45.91	0.01
	Multilocus score	23.35	0.35
	IUGR status x Multilocus score	–35.82	0.21
Sugar consumption (g)	z-BMI 48m	1.72	<0.01
	IUGR status	11.83	0.04
	Sex	4.60	<0.01
	Multilocus score	4.01	0.04
	IUGR status x Multilocus score	–4.56	0.04
Complex carbohydrates consumption (g)	z-BMI 48m	1.31	0.02
	IUGR status	6.34	0.22
	Sex	2.24	0.08
	Multilocus score	0.81	0.65
	IUGR status x Multilocus score	–2.62	0.20
Fiber consumption (g)	z-BMI 48m	0.06	0.48
	IUGR status	0.93	0.22
	Sex	0.26	0.16
	Multilocus score	0.29	0.26
	IUGR status x Multilocus score	–0.39	0.19
Fat consumption (g)	z-BMI 48m	1.07	0.02
	IUGR status	1.71	0.68
	Sex	1.49	0.14
	Multilocus score	0.03	0.98
	IUGR status x Multilocus score	–0.37	0.82
Protein consumption (g)	z-BMI 48m	0.80	0.02
	IUGR status	2.45	0.43
	Sex	0.98	0.20
	Multilocus score	0.73	0.50
	IUGR status x Multilocus score	–0.72	0.55

Table 4: Consumption of the different macronutrients by IUGR and non-IUGR children: means (standard deviations)

		Non-IUGR	IUGR	p-values
Total calories		318 (127)	300 (122)	0.44
Carbohydrates	Total (g)	37 (17)	36 (18)	0.70
	Total (%)	48 (15)	48(14)	0.93
	Sugars (g)	21 (10)	20 (11)	0.63
	Sugars (%)	27 (11)	26 (10)	0.69
	Complex (g)	15 (9)	14 (9)	0.84
	Complex (%)	19 (8)	19 (9)	0.56
	Fiber (g)	2 (1)	2 (1)	0.99
	Fiber (%)	2 (2)	2 (2)	0.69
Fat	Total (g)	13 (7)	12 (6)	0.46
	Total (%)	37 (11)	37 (11)	0.95
Protein	Total (g)	12 (5)	11 (4)	0.45
	Total (%)	15 (4)	15 (3)	0.81


Non−IUGR
IUGR
Total calories
Multilocus score
0
200
400
600
800
1.0
2.0
3.0
4.0


Non−IUGR
IUGR
Consumption of sugar (grams)
Consumption of sugar (grams)
0
10
20
30
40
50
60
1.0
2.0
3.0
4.0
Multilocus score
Multilocus score