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From pregnancy to breastfeeding: adequate maternal body mass index is essential to prevent a high body mass index in your children

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Abstract

Objectives: To verify the associations between prenatal and perinatal factors with offspring body mass index (BMI) and the moderator role of maternal BMI in this relationship.

Methods: Cross-sectional study developed with 1,562 children and adolescents aged between 6 and 17 years, as well as their mothers, from southern Brazil. The prenatal and perinatal factors, weight, and height for the calculation of maternal BMI were self-reported. For the calculation of BMI, weight and height of the child/adolescent were measured on an anthropometric scale with a coupled stadiometer. Linear regression models were used for the moderation analysis. All analyzes were adjusted for the mother's and child's age, sex, sexual maturation, skin color/race, and educational level.

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Physiotherapy and Dance, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil **Results:** cesarean as type of delivery (β =0.66; 95% CI=0.22 1.04; p=0.002) and pregnancy complications (β =0.60; 95% CI=0.15 1.04; p=0.002) were positively associated with offspring BMI. Schoolchildren who were breastfed for 4–6 months showed –0.56 kg/m² of BMI (95% CI=–1.06– 0.06; p=0.02). Birth weight was also associated with BMI, with low weight being inversely (β =–0.59; 95% CI=–1.03– 0.15; p=0.008), while overweight was positively related (β =0.84; 95% CI=0.081.60; p=0.02). The moderation analysis indicated a positive interaction between the mother's BMI and cesarean, pregnancy complications, and smoking with the offspring's BMI. On the other hand, there was an inverse association between breastfeeding from 7 to 12 months and the offspring BMI, only in mothers with high BMI.

Conclusions: Adequate maternal BMI is essential to prevent a high BMI in their children, especially when considering the influence of prenatal and perinatal risk factors.

Keywords: adiposity; perinatal factors; prenatal factors; youth.

Introduction

The rising prevalence of obesity and its related metabolic complications in childhood have driven the attention for the underlying factors that may be associated [1]. Beyond the determining role of genetics and lifestyle, prenatal and perinatal factors have been considered important aspects of the development of these disorders [2, 3]. The environment before birth or in the first years of life may lead to the long-term control of homeostasis and tissue physiology, resulting in adaptative responses associated with several diseases in adulthood [4].

The early risk factors for obesity include prenatal and perinatal aspects, in which prenatal factors contained diseases and complications developed during pregnancy, as well as habits adopted by the mother, such as smoking and drinking, while perinatal factors concern the delivery characteristics, like the type of delivery, birth weight, and acute fetal distress and breastfeeding [5]. A recent

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systematic review indicated that maternal smoking during pregnancy increases the odds of the children becoming overweight by 33 and 55% for obesity [6]. Children and adolescents who were born by cesarean delivery and had a short duration of breastfeeding are also at increased risk of obesity [7, 8]. Concerning birth weight, both low and high [3] are related to obesity and metabolic complications.

In this context, the influence of maternal obesity, in both pre-pregnancy and pregnancy plays an essential role, as may cause some obstetric and perinatal complications. For instance, obese women are more susceptible to pregnancy impairments and present increased cesarean delivery rates [9]. Small for gestational ages (a baby who is smaller than the usual amount for the number of weeks of pregnancy) [10], fetal malformations, and preterm birth are the conditions that the offspring of obese mothers are at increased risk [11, 12]. Also, a maternal pre-delivery body mass index (BMI) of 27.16 kg/m² was suggested as a cut-off for predicting offspring over weight/obesity at the age of 2 years among residents of a suburb in Taiwan [13]. Indeed, not only obesity during pregnancy but also high prepregnancy BMI contributes to excess adiposity and cardiometabolic risk in the offspring during childhood [14].

Therefore, in order to provide a healthy prenatal and perinatal environment for the offspring, as well as to protect the newborn from a high BMI, mothers should present an appropriate BMI before pregnancy and during the gestation period. The relationship between prenatal and perinatal factors with both mother's BMI and offspring BMI have been previously described. However, the interactions between these factors are not fully understood. Taking this aspect into consideration, our study intends to fill a gap in the literature by estimating the point from which maternal BMI may exert a deleterious influence in the relationship between prenatal factors (pregnancy complications, and alcohol and smoking during pregnancy) and perinatal factors (type of delivery, birth weight and breastfeeding) with offspring BMI. Thus, the present study aimed to verify the associations between prenatal and perinatal factors with offspring BMI and the moderator role of maternal BMI in this relationship.

Subjects and methods

Design and study sample

This is a cross-sectional study carried out with 1,562 children and adolescents (44.8% boys) aged between 6 and 17 years, as well as their mothers. Participated students selected by conglomerate from 19 public and private schools, from urban and rural areas in Santa Cruz

do Sul, Rio Grande do Sul, Brazil. Schoolchildren's Health Research started in 2004 in this city and indicated the number of schools (n=50) and students (n=17,688) enrolled. A sample size calculation was done considering the population density of schoolchildren in all regions of the city (south, north, east, west, center) including private and public (municipal and state) schools, which were subsequently randomly selected and invited to form a cohort. For the present study data were collected in 2016 and 2017. The schoolchildren's mothers or legal guardians signed free and informed consent forms, in which it was mentioned that they should answer a questionnaire, authorizing the participation of their children. This study was approved by the Human Research Ethics Committee of the University of Santa Cruz do Sul (UNISC) under certificate number 1.498.305.

The sample size calculation was done through to the software G^* Power version 3.1. A weak effect size (F^2 =0.02), the statistical power of 0.98, and alpha of 0.05, with 9 predictors were considered. The minimum number of participants was established as 1,417. However, to avoid probably difficulties with sample loss, an increase of 10% was assumed, totaling 1,558.

Measurements

All measurements were done at the facilities of the university, carried out by a team of trained researches.

Children's and adolescent's evaluations

Height and weight were evaluated using an anthropometric scale with a coupled stadiometer (Filizola[®]). BMI was determined by dividing weight (kg) by height (m) squared, which has been widely used as an indicator of an excess of body weight [15]. BMI was categorized into underweight, normal weight, overweight, or obesity according to Onis et al. [16] and the sample description we have used underweight/normal weight, and overweight/obesity. Tanner's criteria were considered to determine sexual maturation [17], which considers images of the development of genital stages, and pubic hair. Participants were asked to indicate the image accordingly to their current stage. Five sexual maturation stages were considered and then, categorized into prepubertal (stage I), initial development (stage II), continuous maturation (stages III and IV), or matured (stage V).

A self-reported questionnaire was used to determine skin color/ race and participants should have indicated one of the following options: white, black, brown/mulatto, indigenous, or yellow.

Mother's evaluations

Information regarding the perinatal factors and mother's characteristics were obtained by a self-reported questionnaire answered by mothers, according to the following questions and answers options: (1) Which was the type of delivery? Cesarean/vaginal; (2) Did you have any complications during pregnancy? Yes/no; (3) For how many months did you exclusively breastfeed? For analysis purposes, the answers were categorized (never, 1–3 months, 4–6 months, 7–12 months, or more than one year); (4) Which was the birth weight of your children? For analysis purposes, the answers were categorized (adequate, underweight, or overweight) [18]; (5) During pregnancy did you drunk? Yes/no; (6) During pregnancy did you smoke? Yes/no; (6) what was the gestational age? For analysis purpose, the answers were Table 1: Offspring characteristics.

categorized as preterm (<37 completed weeks); term (37 to <42 completed weeks), or post-term (>42 completed weeks; (7) How old were you during pregnancy? Also, mothers were asked to report their weight and height for BMI calculation, as well as education level (Illiterate/incomplete 1st to 4th grade, incomplete 5th to 8th grade, high school incomplete, incomplete higher education, or complete higher education).

Statistical analysis

Descriptive data are presented as means, standard deviation, and frequency. The data normality was tested through Kolmogorov– Smirnov test. Linear regression models were performed to determine the associations between perinatal factors with children's and adolescent's BMI.

Moderation analyses were applied using the PROCESS macro for the Statistical Package for Social Sciences (SPSS) version 24.0 (IBM Corp. Armonk, NY, USA), through multiple linear regression models. The following associations were tested: (1) Mother's BMI with offspring BMI; (2) Perinatal and prenatal factors (the type of delivery, pregnancy complications, breastfeeding duration, and smoking during pregnancy, tested in different models) with offspring BMI; and (3) interaction between perinatal factors X mother's BMI with offspring BMI. The Johnson-Neyman technique [19] was used to assess whether mother's BMI moderated the relationship between prenatal and perinatal factors with offspring BMI, to indicate regions of significance. Through this technique it is possible to highlight, in the context of the present study, specific values of mother's BMI (classified according to tertiles in low, medium, and high) in which the significant relationship between prenatal an perinatal factors with offspring BMI appears or disappears.

All analyses were adjusted for children's and mother's age, sex, sexual maturation, skin color/ethnicity, and education level. The probability value p<0.05 was considered to be significant for all analyses.

Results

Table 1 presents the descriptive characteristics of the sample. The majority of the sample was composed of girls (55.2%), and 43.5% of the children and adolescents presented overweight/obesity. Concerning perinatal factors, 27.4% of the mothers reported that have never breastfed their children, and 32.3% exclusive breastfeeding during 4–6 months.

The mother's characteristics are presented in Table 2. Almost half of the sample had cesarean as a type of delivery (49.8%) and 25.1% presented pregnancy complications. Finally, 57.1% of the mothers were classified as overweight/obese.

The association between perinatal factors with offspring BMI is presented in Table 3. Cesarean as a type of delivery and pregnancy complication were positively associated with offspring BMI. Also, children and adolescents that were

	Children (n=729)	Adolescents (n=833)	Total (n=1,562)
Characteristics		Mean (SD)	
Age, years	9.14 (1.43)	13.79 (1.51)	11.62
			(2.75)
Height, m	1.39 (0.10)	1.61 (0.09)	1.51 (0.14)
Weight, kg	38.02	56.96 (13.41)	48.12
	(11.51)		(15.71)
BMI, kg/m²	19.19	21.67 (4.00)	20.51
	(3.89)		(4.14)
	n (%)		
Sex			
Boys	331 (45.4)	369 (44.3)	700 (44.8)
Girls	398 (54.6)	464 (55.7)	862 (55.2)
Body mass index			
Normal weight	364 (49.9)	518 (62.2)	882
			(56.5%)
Overweight/obese	365 (50.1)	315 (37.8)	680
			(43.5%)
Exclusive breastfeeding	duration		
Never	181 (24.8)	247 (29.7)	428 (27.4)
1-3 months	78 (10.7)	114 (13.7)	192 (12.3)
4-6 months	270 (37.0)	253 (28.2)	505 (32.3)
7–12 months	96 (13.2)	110 (13.2)	206 (13.2)
More than 1 year	104 (14.3)	127 (15.2)	231 (14.8)
Birth weight, kg			
Underweight	182 (25.5)	220 (26.4)	402 (26.4)
Overweight	51 (7.2)	57 (7.0)	108 (7.1)
Adequate	480 (67.3)	533 (65.8)	1,013 (65.5)
Gestational age			
Preterm	60 (8.6)	697 (88.0)	140 (9.4)
Term	629 (90.0)	80 (10.1)	1,326
			(88.9)
Post-term	10 (1.4)	15 (1.9)	25 (1.7)
Maturational stage			
Pre-pubertal	304 (41.7)	17 (2.0)	321 (20.6)
Initial development	242 (33,2)	102 (12.2)	344 (22.0)
Continuous matura-	162 (22.3)	582 (69.8)	744 (47.7)
tion (stage III and IV)			
Maturated	21 (2.9)	132 (15.8)	153 (9.8)
Skin color/Ethnicity			
White	617 (84.6)	658 (79.0)	1,275 (81.6)
Black	43 (5.9)	53 (6.4)	96 (6.1)
Brown/mulatto	67 (9.2)	111 (13.3)	178 (11.4)
Others	2 (0.2)	11 (0.13)	13 (0.8)

SD, standard deviation.

breastfed during 4-6 months presented -0.56 kg/m² of BMI. Birth weight was also associated with BMI, in which underweight was inversely associated while overweight was positively related.

Additionally, we sought to test the influence of mother's BMI in the associations between prenatal and perinatal

Table 2: Mother's characteristics in data collection (2016/2017)

 and during pregnancy.

 Table 3: Association between perinatal factors and the mother's body mass index with offspring body mass index.

Characteristics	Mean (SD)
Age, years ^a	39.31 (7.26)
Age during pregnancy, years	27.2 (6.84)
	n (%)
Body mass index ^a	
Normal weight	590 (42.9)
Overweight/Obese	784 (57.1)
Type of delivery	
Vaginal	744 (50.2)
Cesarean	737 (49.8)
Pregnancy complication	
Yes	372 (25.1)
No	1,112 (74.9)
Alcohol consumption during pregnancy	
Yes	1,094 (91.4)
No	103 (8.6)
Smoking during pregnancy	
Yes	108 (11.3)
No	851 (88.7)
Education level ^a	
Illiterate/incomplete 1st to 4th grade	90 (5.8)
Incomplete 5th to 8th grade	375 (24.0)
High school incomplete	277 (17.7)
Incomplete higher education	569 (36.4)
Complete higher education	251 (16.1)

^aCharacteristics in data collection (2016/2017).

factors with offspring BMI (Table 4). Data indicated interactions between mother's BMI with the type of delivery, pregnancy complication, exclusive breastfeeding duration of 7–12 months, and smoking during pregnancy with offspring BMI.

Figure 1 shows the point from which the mother's BMI exerts a deleterious influence in the relationship between prenatal and perinatal factors with offspring BMI. Type of delivery (cesarean) showed a positive association with offspring BMI, only for the mother with a BMI greater than 26.78 kg/m². Similarly, pregnancy complications and smoking during pregnancy along with high mother's BMI (higher than 27.37 and 28.55 kg/m², respectively) were associated with offspring BMI. Otherwise, it was found an inverse relationship between breastfeeding during 7 and 12 months with offspring BMI only in the mothers with high BMI.

Discussion

The main findings of the present study indicate that perinatal factors, such as type of delivery, pregnancy

	Offspring BMI			
Variables	β	95% CI	p-Value	
Type of delivery				
Vaginal	1			
Cesarean	0.66	0.22 1.04	0.002	
Pregnancy complication				
No	1			
Yes	0.60	0.15 1.04	0.008	
Exclusive breastfeeding of	duration			
Never	1			
1–3 months	-0.14	-0.79 0.51	0.67	
4–6 months	-0.56	-1.06-0.06	0.02	
7–12 months	-0.38	-1.02 0.25	0.23	
More than 1 year	-0.04	-0.66 0.57	0.88	
Birth weight				
Adequate	1			
Underweight	-0.59	-1.03-0.15	0.008	
Overweight	0.84	0.08 1.60	0.02	
Alcohol consumption dur	ing pregnanc	y		
No	1			
Yes	0.35	-0.43 1.13	0.38	
Smoking during pregnan	су			
No	1			
Yes	0.77	-0.02 1.58	0.056	
Mother's BMI				
Normal weight	1			
Over weight/Obese	1.80	1.39 2.21	<0.001	

BMI, body mass index. All analyses were adjusted for children's and mother's age, sex, sexual maturation, skin color/ethnicity, and education level. Bold denotes significant associations.

complication, breastfeeding duration, and birth weight are associated with offspring BMI. The mother's BMI was also positively associated with offspring BMI. Also, it was found an interaction between mother's BMI with the type of delivery, pregnancy complications, exclusive breastfeeding duration, and smoking during pregnancy and offspring BMI. We highlight that, to the best of our knowledge, this is the first study to determine the point from which a mother's BMI exerts a deleterious influence on offspring BMI when considering these perinatal factors.

Maternal healthy or unhealthy conducts and conditions during pregnancy and delivery are closely related to the offspring's health [6, 20]. Our findings indicated that offspring born by cesarean presented higher BMI. This is in accordance with a study developed in the northeast of Brazil, which showed that children born from cesarean had a two times higher risk of obesity, that the ones born by vaginal delivery [21]. This study also showed a similar prevalence of cesarean delivery (47.6%) than ours (49.8%) (16), about three times higher than the guidelines by the **Table 4:** Moderation of the mother's body mass index in the relationship between prenatal and perinatal factors and children's and adolescent's body mass index.

	Offspring body mass index		
	β	CI (95%)	p- Value
Model 1			
Mother's BMI	0.14	0.09 0.19	<0.001
Type of delivery			
Vaginal	1		
Cesarean	-2.02	-3.99	0.04
		(-0.05)	
Mother's BMI × type of delivery	0.08	0.01 0.16	0.01
Model 2			
Mother's BMI	0.02	-0.08 0.13	0.66
Pregnancy complication			
No	1		
Yes	-2.88	-5.07	0.01
		(-0.69)	
Mother's BMI $ imes$ pregnancy	0.12	0.04 0.20	<0.001
complication Model 3			
	0.23	0 1 7 0 20	(0.001
Mother's BMI	0.25	0.17 0.30	<0.001
Exclusive breastfeeding duration Never	1		
		2 00 2 72	0.00
1–3 months	0.41		0.80 0.41
4–6 months 7–12 months	5.07	-1.46 3.52 2.03 8.11	
			<0.001
More than 1 year	0.13	-3.24 3.51	0.93
Mother's BMI \times 1–3 months	-0.005	-0.12 0.11	0.93
Mother's BMI \times 4–6 months Mother's BMI \times 7–12 months	-0.05	-0.14 0.03	0.25 <0.001
Mother's BMI × 7–12 months	-0.20	-0.31 (-0.08)	<0.001
Mothor's PML y more than 1 year	0 002	-0.25 0.53	0.49
Mother's BMI × more than 1 year Model 4	-0.003	-0.25 0.55	0.49
Mother's BMI	0.21	0.15 0.27	<0.001
Birth weight	0.21	0.15 0.27	10.001
Adequated	1		
Underweight	3.20	-0.52 6.93	0.00
Overweight		-0.52 0.95	0.09 0.19
Mother's BMI × underweight	-0.07	-0.20 0.06	0.19
Mother's BMI × overweight	-0.07	-0.10 0.04	0.28
Model 5	-0.05	-0.10 0.04	0.42
Mother's BMI	0 1 1	-0.15 0.37	0.41
Alcohol consumption during	0.11	0.19 0.97	0.41
pregnancy			
No	1		
Yes		-2.44 5.30	0.46
Mother's BMI × alcohol consumption		-0.09 0.17	0.56
during pregnancy	0.04	0.09 0.17	0.50
Model 6			
Mother's BMI,	0.66	0.42 0.89	<0.001
Smoking during pregnancy	0.00	0.72 0.07	
No	1		
Yes	-6.45	-10.06	<0.001
	5175	(-2.85)	
		(2.03)	

Table 4: (continued)

	Offsprir	Offspring body mass index		
	β	CI (95%)	p- Value	
Mother's BMI × smoking during pregnancy	0.25	0.12 0.38	<0.001	

BMI, body mass index. All analyses were adjusted for children's and mother's age, sex, sexual maturation, skin color/ethnicity, and education level. Bold denotes significant associations.

World Health Organization [22], who recommends that the rate of cesarean in any region of the world should not exceed 15%. Although this reality is not faced only in Brazil, countries like India and Mexico present similar data, showing cesarean rates at 46 and 40% respectively [23, 24]. Further, it is worth mentioning that the consequences of cesarean may be observed until adulthood, increasing the risk of obesity by 11% and for type 2 diabetes by 46% [25].

Concerning breastfeeding duration, results showed that 32.3% of the sample was exclusively breastfed during 4-6 months, 13.2% during 7-12 months, and 14.8% for more than one year, indicating that a higher proportion of mothers comply with the recommendation of breast milk as the exclusive nutrient source for the first six months of life [26]. These data indicate a positive scenario, compared to data from low and middle-income countries, showing that only 37% of the children younger than 6 months are exclusively breastfed [27]. The observed association between breastfeeding during 4 to 6 months with lower BMI is also supported by available evidence, in which longer breastfeeding duration was associated with a 13% reduction in the prevalence of overweight or obesity, even after controlling for potential confounders such as socioeconomic status and maternal BMI [27]. Besides, breastfeeding reduces the risk for hypertension and diabetes, allergy and respiratory infection to children from birth, generating an affective bond between mother and son [28]. Another aspect to be considered is that the breast milk prepares the child for the gradual introduction of complementary food. Thus, the timing of the introduction of complementary feeding may be associated with being overweight during childhood, with evidence showing that this introduction before 4 months was associated with a higher BMI in childhood [29].

Concerning birth weight, 65.5% of children and adolescents in our sample presented adequate birth weight (ranging between 3 and 3.999 kg), as recommended by the

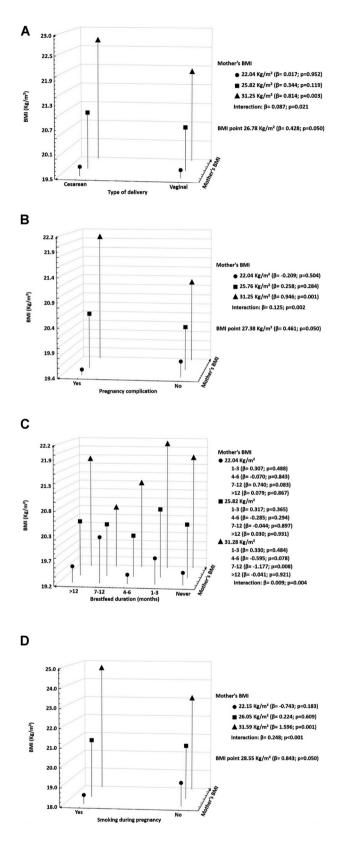


Figure 1: The role of maternal body mass index in the relationship between perinatal factors (A-type of delivery; B-pregnancy complication; C-breastfeed duration; D-smoking during pregnancy) with children's and adolescent's body mass index.

World Health Organization 18. Indeed, this perinatal aspect is another early risk factor for obesity, as shown in the present study a higher birth weight was linked to higher BMI, which is in agreement with the previous literature [3, 30].

It is well established that the mother's obesity is an important intervenient factor for the prenatal and perinatal environment. For example, the duration of breastfeeding is usually shorter in obese women than in normal-weight women [20] and the risk of cesarean delivery is higher [31]. Given also that, the association between mother's and offspring BMI found in the present study is already established, we intend to understand how prenatal and perinatal factors interact with mother's obesity to exert an influence on offspring BMI. Our data indicated that cesarean type of delivery was associated with offspring BMI. only for the mother with a BMI higher than 26.78 kg/m². Both conditions, pregnancy complications, and smoking during pregnancy were associated with offspring BMI, but only for the mothers with a BMI higher than 27.37 and 28.55 kg/m², respectively. Therefore, these prenatal and perinatal factors exert an influence on offspring BMI, at a certain level of maternal BMI. Also, our findings showed an inverse relationship between breastfeeding during 7-12 months with offspring BMI only in the mother's with high BMI, that is, if the breastfeeding happens during this period, even if the mother present excess of body weight the children/adolescent will be protected from a high BMI.

In this context, maternal nutrition before pregnancy may contribute to the establishment of the epigenetic profiles in the fetus leading to increased susceptibility to adiposity and metabolic disorders later in life [32]. Therefore, the exposure to certain factors, including the ones investigated in the present study may result in epigenetic changes modulating fetal gene expression with long-term consequences [33]. Present findings are also supported by the theory of the Developmental Origins of Health and Disease, which is based on the concept that early life environment exposures exert a relevant influence on the predisposition to disease risk development later in life [34].

We emphasize the relevance of these findings, as according to our knowledge no studies are investigating the moderator role of maternal BMI on the aforementioned associations, and mainly the specific point from which BMI may exert a deleterious effect. Obesity is a multifactorial disease, related to genetics, environmental factors,

All analyses were adjusted for children's and mother's age, sex, sexual maturation, skin color/ethnicity, education level. Type of delivery: Reference category = vaginal. Pregnancy complication and smoking during pregnancy: reference category = no Breastfeed duration: reference category = never.

lifestyle habits, such as sedentary behavior, low levels of physical fitness, and inappropriate sleep [35]. Beyond these aspects, it is essential to understand the prenatal and perinatal factors there are involved, to promote strategies to prevent obesity since the first years of life.

The present study has some limitations. Some possible confounding factors were not included, such as the mother's physical activity, and if the mothers had prenatal care. There was no information about maternal BMI at the time of her child's birth, which could be an important intervenient factor in the analyzed variables. We also did not measure the offspring diet. The prenatal and perinatal factors were self-reported, which could increase the chance of memory bias, although a lot of evidence develops the same protocol [23, 25]. Finally, the cross-sectional data did not allow for causal inferences. Otherwise, some important strengths must be acknowledged, such as the representative sample of schoolchildren, and considering many prenatal and perinatal factors that may be associated with offspring BMI. Considering BMI as a simple and inexpensive measure to determine excess body weight, which has been shown as more accurate to predict cardiovascular disease risk compared with measures of adiposity, such as fat mass index and percentage of body fat [15]. In addition, the present results fill a gap in the literature, by indicating the point from which mother's BMI exerts a deleterious influence on offspring BMI when considering prenatal and perinatal factors. We highlight that future studies should be developed with other samples in order to reinforce that present findings.

In conclusion, type of delivery, pregnancy complication, breastfeeding duration, and birth weight are associated with offspring BMI. Furthermore, the mother's BMI is a moderator in the association between type of delivery, pregnancy complications, exclusive breastfeeding duration, and smoking during pregnancy with offspring BMI. Therefore, an appropriate maternal BMI throughout life is essential to prevent a high BMI in their children, especially when considering the influence of early risk factors.

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Competing interests: Authors state no conflict of interest. **Informed consent:** Informed consent was obtained from all individuals included in this study.

Ethical approval: This study was approved by the Human Research Ethics Committee of the University of Santa Cruz do Sul (UNISC) (number 1.498.305) and followed the resolution 466/2012 of the National Council of Health in Brazil. The informed consent form for participation in this study was provided by the legal guardian of the participants.

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