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Article

Added Sugar Intake Is Associated with Altered Plasma miR-143-5p and miR-223-3p Expression and Fasting Glucose Concentrations in Children from Mexico City

Alejandro Castañeda-López [1], María F. Velázquez-Rodríguez [2], Leonor Jacobo-Albavera [3], María Fernanda Pérez-Hernández [1,4], José de Jesús Peralta-Romero [1], Sofía Barragán-Vázquez [5], Claudia I. Ramírez-Silva [6], Andrés Rocha-Aguado [7], Fengyang Huang [8], Fausto Sánchez-Muñoz [9], Miguel Cruz [1] and Adrián Hernández-Díazcouder [1]*

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Abstract

Background: Childhood obesity is a growing health concern and added sugar intake has been associated with increased obesity risk. Emerging evidence suggests that miR-143-5p and miR-223-3p are involved in metabolic regulation. Therefore, this study evaluated their plasma expression according to added sugar intake and their association with cardiometabolic risk parameters. **Methods:** In this cross-sectional study, 91 children aged 6 to 12 years were included (44 normal weight and 47 with overweight/obesity). Anthropometric, and clinical parameters, and dietary added sugar consumption were analyzed. RT-qPCR was performed to assess the expression of miR-143-5p and miR-223-3p in plasma. **Results:** Compared with low added sugar intake, high added sugar intake was associated with lower plasma miR-143-5p expression and higher miR-223-3p expression. Both miR-143-5p and miR-223-3p expression levels were higher in children with overweight/obesity and insulin resistance than in their respective counterparts. In adjusted analyses, neither miRNA was associated with cardiometabolic risk parameters. However, added sugar intake was positively associated with miR-223-3p expression ($\beta = 0.188$, 95% CI: 0.022, 0.354) and glucose levels ($\beta = 1.944$, 95% CI: 0.133, 3.755). **Conclusions:** High added sugar intake was associated with lower miR-143-5p expression, higher miR-223-3p expression, and increased fasting glucose concentrations in school-aged children, suggesting early alterations in metabolic regulation.

Keywords: added sugar; circulating microRNAs; childhood obesity; cardiometabolic factors; insulin resistance; miR-143-5p; miR-223-3p

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1. Introduction

Childhood obesity represents a growing global health challenge, given that overweight and obesity during childhood frequently persist into adulthood [1]. Epidemiological evidence has shown a marked worldwide rise in the number of children living with overweight and obesity. In particular, the prevalence of obesity among children aged 5 to 14 years increased more than threefold globally, from 2.0% in 1990 to 6.8% in 2021, and projections indicate that it could reach 15.6% by 2050 [2]. In addition, in Mexico, obesity has been reported in 18.8% of children between 5 and 11 years of age [3]. Childhood obesity is linked to the early development of cardiovascular risk factors, including hypertension, dyslipidemia, insulin resistance, and glucose intolerance [4].

Among the dietary factors associated with this condition, higher added sugar intake has been linked to weight gain and an increased risk of obesity in children [5–7]. In Mexico, the 2020-2022 National Health and Nutrition Survey (ENSANUT) [8] reported that added sugar intake among children aged 5 to 11 years was approximately 50 g/day, representing 12% of total energy intake. In addition, 66.9% of school-aged children consumed more than 10% of their daily energy intake from added sugar [8]. In this context, the World Health Organization (WHO) recommends reducing free sugar intake to less than 10% of total energy intake.

Emerging studies suggest that epigenetic factors such as microRNAs (miRNAs) play a role in metabolic regulation. MiRNAs are small, endogenous, single-stranded non-coding RNAs that play an important role in the post-transcriptional regulation of gene expression through their interaction with the 3′ untranslated region (3′UTR) of target messenger RNAs (mRNAs) [9]. miRNAs are released from cells into the circulation [10], where extracellular circulating miRNAs contribute to the regulation of whole-body metabolism through intercellular communication [11]. Circulating miRNAs have gained attention as novel biomarkers of obesity and related metabolic disorders [11,12].

Several studies have highlighted the important role of miR-143-5p in lipid metabolism, adipogenesis, and IR [13]. For instance, miR-143-5p is related to the induction of IR by regulating insulin signaling [14]. Also, this miRNA promotes lipid accumulation in adipocytes [15]. miR-223-3p is implicated in the inflammatory response [16] and has been linked to metabolic alterations related to obesity [17]. Moreover, experimental studies have reported that these miRNAs may be regulated by high-carbohydrate diets [18,19]. In this context, assessing the expression of these miRNAs in plasma is a minimally invasive strategy to investigate molecular alterations associated with dietary factors and obesity in children. Thus, the present study aimed to evaluate the association between added sugar intake and plasma expression of miR-143-5p and miR-223-3p in children, and whether the expression of these miRNAs was associated with cardiometabolic risk parameters.

2. Materials and Methods

Participants in the Study

The study population consisted of 91 unrelated children from Mexico City, aged 6 to 12 [44 normal weight (NW) and 47 with overweight and obesity (Ow/Ob)]. Participants were recruited at Family Medicine Unit No. 23 of the Instituto Mexicano del Seguro Social in Mexico City. The study was conducted to evaluate the association of added sugar intake with miRNA expression, obesity, and cardiometabolic risk factors. Children presenting with acute infections, chronic diseases, participation in a weight loss program, or fasting plasma glucose levels exceeding 126 mg/dL were excluded from the study.

Ethics Statement

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The study was approved by the Ethics Committee of the Instituto Mexicano del Seguro Social (CONBIO-ETICA-09-CEI-009-20160601; approval number: R-2024-785-061) on 23 October 2024 and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participants, and assent was obtained from the participating children.

Anthropometric Assessment

According to standardized protocol, the following anthropometric measurements were performed to ensure accuracy, consistency, and data reliability. The procedure was performed by trained personnel with expertise in anthropometric techniques, using calibrated instruments under controlled conditions.

Each child participant underwent measurement of body weight, height, waist circumference, and hip circumference in a single session at the beginning of the study. Body weight was measured using a digital scale with a precision of 100 g (Seca, Hamburg, Germany). Children were weighed wearing light clothing and no shoes, standing upright in the center of the scale platform, maintaining a straight posture, and remaining still during the reading. For height measurement, a portable device with 0.1 cm resolution was utilized (Seca 225, Hamburg, Germany). The child stood barefoot, heels together, back straight, and head aligned in the horizontal plane (Frankfurt plane), ensuring contact between the heels, buttocks, scapulae, and head with the vertical surface. Waist circumference was measured using a 0.1 cm precision flexible tape measure, resistant to stretching, placed around the abdomen just above the upper edge of the iliac crest, and recorded at the end of a gentle expiration. The child stood upright in a relaxed posture, with arms hanging loosely at the sides and feet slightly apart. Hip circumference was measured with the same type of tape, placed horizontally around the most prominent part of the buttocks, ensuring the tape was level and did not compress the soft tissue. As with other measurements, the child stood still and relaxed throughout the procedure. Each measurement was recorded immediately on an individual data collection sheet and double-checked to ensure accuracy. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m2). BMI values were converted into age- and sex-specific percentiles. The data were then processed using the WHO Anthro Plus software and evaluated according to the World Health Organization (WHO) growth standards [20]. Children were classified according to the WHO BMI-for-age reference for children and adolescents aged 5 to 19 years using BMI-for-age z-scores. Participants were categorized as having normal weight when their BMI-for-age z-score was ≥ −2 and ≤ +1 ZSD, overweight/obesity when it was > +1 Z [20].

Biochemical Assessment

In the morning, after an 8 h fast, venous blood samples were collected to determine the levels of glucose, insulin, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (Total CHO), and triglycerides (TG). Serum concentrations of glucose, LDL-C, HDL-C, Total CHO, and TG were measured using an enzymatic colorimetric method on the ILab 350 Clinical Chemistry System (Instrumentation Laboratory IL, Barcelona, Spain). Fasting insulin levels were measured using the Elecsys Insulin Assay on the Cobas e411 immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Fasting glucose and insulin concentrations were used to calculate the homeostasis model assessment of IR (HOMA-IR) using the following formula: fasting insulin (μU/mL) × fasting glucose (mg/dL) / 405. IR was defined as HOMA-IR ≥3.16, based on a cutoff previously proposed for children and adolescents with obesity [21,22].

Assessment of Added Sugar Intake

A semi-quantitative food frequency questionnaire (FFQ) was used to assess dietary added sugar intake over the past month [23]. The FFQ was designed based on the Mexican ENSANUT for school-aged children, developed by the National Institute of Public Health, as a reference. Additionally, for

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some newly incorporated foods considered relevant to this population and study, reference portion sizes were estimated from 24-hour dietary recalls obtained from ENSANUT for school-aged children, and all portions were expressed using household measures. For the estimation of added sugars, an algorithm adapted from the methodological approach proposed by Louie et al. [24], as modified by our research group*, was applied using information from the Mexican Food Composition Database (BAM) [25]. The FFQ was analyzed following the same methodological criteria used in the analysis of grams and nutrients in the ENSANUT FFQ [26]. Children were classified as having high added sugar intake when consumption was ≥50 g/day and low added sugar intake when consumption was <50 g/day. This cutoff was defined based on a 2,000 kcal diet, which corresponded approximately to the average total energy intake of the study population.

miRNA Expression in Plasma

RNA extraction from plasma was performed using the miRNeasy Serum/Plasma Kit (Qiagen, Waltham, Hilden, Germany), according to the manufacturer's instructions. During the RNA purification step, the same amount of cel-miR-39 spike-in control (Qiagen, Hilden, Germany) was added according to the provider's recommendations and a previous publication [27]. The miRNA isolated from plasma was immediately converted to cDNA, as described below.

miRNAs were determined using two-step RT-qPCR with RT-primer specific assay in combination with TaqMan probes: hsa-miR-143-5p (Assay ID: 002146) and hsa-miR-223-3p (Assay ID: 002295) (Applied Biosystems; CA, USA). Each RT-reaction used 1.5 µL from the 14 µL eluted miRNA using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems; CA, USA). The RT-reaction program consisted of 30 minutes at 16 °C, 30 minutes at 42 °C, and 5 minutes at 85 °C.

The 1 µL of RT reaction was amplified in 10 µL reactions. PCR cycling conditions were initial denaturation at 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s, at 60 °C for 60 s, and at 72 °C for 1 s. PCR was performed using a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with the Maxima Probe/ROX qPCR Master Mix (Thermo Scientific, Waltham, MA, USA). Relative miRNA expression levels were normalized with Ct values of cel-miR-39, and values were calculated using the $2^{-\Delta\Delta Ct}$ formula. All Ct values for cel-miR-39 ranged from 20 to 22 cycles for total plasma miRNA isolations.

Statistical Analysis

Differences in population characteristics were evaluated using Student's t-test for normally distributed variables and the Mann–Whitney U test for non-normally distributed variables. Categorical variables were compared using Pearson's chi-squared test. The distribution of continuous variables was assessed using the Shapiro–Wilk test.

Exploratory associations between miRNAs expression, added sugar intake, and cardiometabolic risk parameters were examined using Spearman's correlation coefficients, as well as Student's t-test.

Linear regression models were subsequently used to examine adjusted associations. Models adjusted for sex, age, BMI and total energy intake assessed associations between (i) miRNA expression (dependent variable) and cardiometabolic parameters, as well as (ii) between added sugar intake (independent variable) and miRNA expression or cardiometabolic outcomes. To facilitate interpretation, added sugar intake was modeled as a continuous variable, with effect estimates expressed per 25 g/day increment in intake. For all analyses, a two-tailed p-value < 0.05 was considered statistically significant. All analyses were conducted using Stata software (version 19.5; StataCorp LLC, College Station, TX, USA).

3. Results

Study Population Characteristics

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Table 1 shows the children's general characteristics and health outcome variables. The mean age was 9.197 ± 1.802 years. Males comprised 52% of the sample, with a higher proportion among children with overweight/obesity (~62%). BMI, BMI Z-score, waist and hip circumferences, insulin, HOMA-IR, total CHO, and TG levels were significantly higher in children with overweight/obesity compared with those with normal weight ($P < 0.05$), whereas HDL-C levels were lower in children with overweight/obesity ($P < 0.001$). No differences were observed in the participant's age, glucose levels, LDL-C, and total energy or added sugar intake ($P > 0.05$).

Table 1. Demographic, clinical, and cardiometabolic parameters.

	Total (*n* =91)	NW (*n* = 44)	Ow/Ob (*n* = 47)	P-value
Age (years)	9.197 ± 1.802	8.931 ± 1.822	9.446 ± 1.766	0.174[a]
Sex, n(%)				
Male vs Female	47 (51.650)	18 (40.910)	29 (61.700)	0.047[b]
BMI (kg/m²)	18.653 (16.005, 25.716)	16.105 (15.422, 17.400)	25.414 (23.804, 28.268)	<0.001[c]
BMI Z-score	1.160 (0.140, 2.160)	0.125 (-0.270, 0.475)	2.160 (1.930, 2.370)	<0.001[c]
Waist (cm)	70 (59, 85)	60 (55, 63)	85 (79, 92)	<0.001[c]
Hip (cm)	81 (70, 93)	70 (65, 77)	93 (84, 100)	<0.001[c]
CHO (mg/dL)	156.875 ± 27.853	150.047 ± 20.807	163.268 ± 32.044	0.022[a]
HDL-C (mg/dL)	45.240 ± 9.723	51.325 ± 8.669	39.544 ± 6.794	<0.001[a]
LDL-C (mg/dL)	80.657 ± 27.454	75.061± 22.872	85.895 ± 30.458	0.059[a]
TG (mg/dL)	145.500 (84.600, 221.700)	91.500 (69.800, 145.400)	196.900 (144.800, 240.500)	<0.001[c]
Glucose (mg/dL)	87.973 ± 8.703	87.629 ± 7.811	88.295 ± 9.536	0.717[a]
Insulin (µU/mL)	13.300 (8.010, 24.800)	8.69 (6.450, 11.615)	23.890 (15.770, 32.920)	<0.001[c]
HOMA-IR	3.066 (1.770, 5.253)	1.820 (1.407, 2.398)	5.006 (3.461, 6.872)	<0.001[c]
≥ 3.16, n(%)	48 (52.750)	9 (20.450)	39 (82.980)	<0.001[b]
Total energy intake (Kcal)	2542.183 ± 799.027	2641.665 ± 857.360	2449.051 ± 737.342	0.252[a]
Added sugar intake (g)	65.103 (39.137, 90.733)	67.566 (45.432, 88.746)	63.282 (37.540, 86.427)	0.356[c]
≥ 50 g, n(%)	54 (59.340)	29 (65.910)	25 (53.190)	0.217[b]

BMI: Body mass index; CHO: Cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides; HOMA-IR: Homeostatic model assessment of IR. Results are expressed as mean ± standard deviation for parametric data or as median (interquartile range) for non-parametric data. a Student's t test; b Chi-square test; c Mann–Whitney test. P values < 0.05 were considered statistically significant.

Expression of miRNAs miR-143-5p and miR-223-3p in Children with High and Low Added Sugar Intake

High added sugar intake was associated with altered plasma miRNA expression in children. As shown in Figure 1A, miR-143-5p expression levels (1.000 ± 0.218 vs 0.775 ± 0.2228; $P < 0.001$) were lower in children with high added sugar intake compared to those with low intake, whereas miR-

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223-3p expression levels (1.000 ± 0.142 vs 1.106 ± 0.162; P = 0.002) were higher in children with high added sugar intake than in those with low intake.

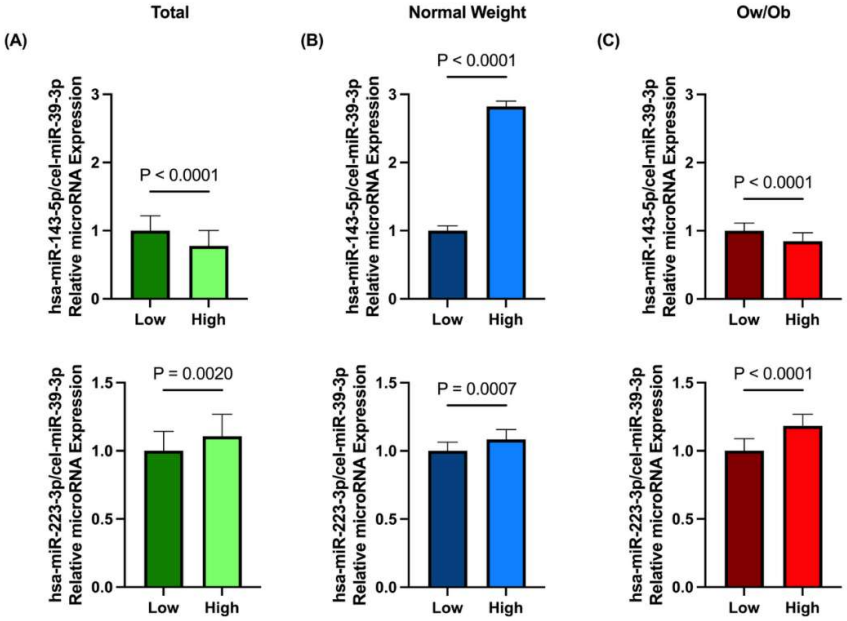


Figure 1. Plasma miRNA expression in children with NW and Ow/Ob stratified by low and high added sugar intake. (A) miR-143-5p and miR-223-3p expression in total children (green bars), (B) miR-143-5p and miR-223-3p expression in children with NW (blue bars), and (C) miR-143-5p and miR-223-3p expression in children with Ow/Ob (red bars). miRNA expression was measured by RT-qPCR using cel-miR-39 as the reference for the $2^{-\Delta\Delta Ct}$ method. Differences were tested using the unpaired t-test. Results are presented as mean ± SD.

Among children with NW, those with high added sugar intake showed higher expression levels of miR-143-5p (1.000 ± 0.072 vs 2.823 ± 0.079; P < 0.001) (Figure 1B) and miR-223-3p (1.000 ± 0.064 vs 1.084 ± 0.072; P < 0.001) (Figure 1B) than those with low added sugar intake. Among children with Ow/Ob, miR-143-5p expression levels (1.000 ± 0.112 vs 0.845 ± 0.124; P < 0.001) were lower in children with high added sugar intake, whereas miR-223-3p expression levels (1.000 ± 0.088 vs 1.184 ± 0.084; P < 0.001) were higher (Figure 1C).

Subsequently, we evaluated miRNA expression levels in children according to nutritional status. Both miR-143-5p (1.000 ± 0.076 vs 5.354 ± 0.118; P < 0.001) and miR-223-3p expression (1.000 ± 0.069 vs 1.280 ± 0.086; P < 0.001) were higher in children with Ow/Ob compared with children with NW (Figure 2A and 2B).

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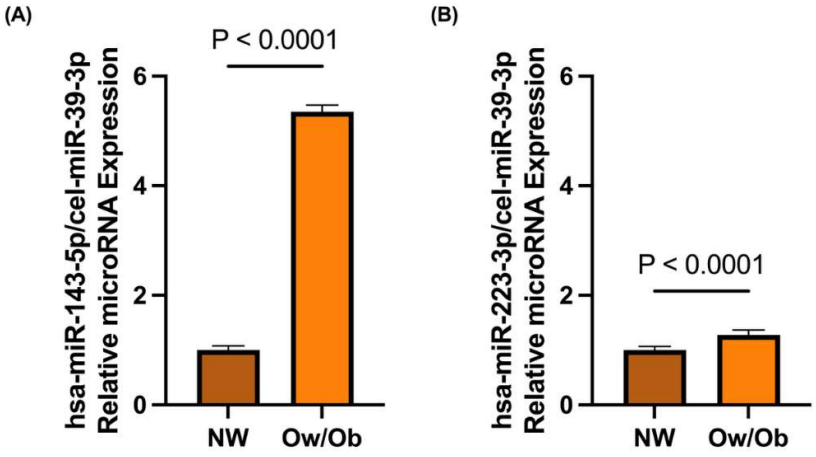


Figure 2. The plasma miRNA expression in children with NW and with Ow/Ob. (A) miR-143-5p expression and (B) miR-223-3p expression. miRNA expression was measured by RT-qPCR using cel-miR-39 as a reference for the $2^{-\Delta\Delta Ct}$ method. Differences were tested by an unpaired t-test. Results are presented as mean ± SD. NW: normal weight; Ow/Ob: Overweight/Obesity.

We further examined whether miRNA expression differed by sex and nutritional status. As shown in Figure 3A, among children with NW, miR-143-5p expression was higher in girls than in boys (1.000 ± 0.075 vs 1.273 ± 0.107; P < 0.001), whereas miR-223-3p expression showed no significant differences (1.000 ± 0.075 vs 0.981 ± 0.076; P = 0.453). Conversely, among children with Ow/Ob, miR-223-3p expression (1.000 ± 0.086 vs 1.203 ± 0.091; P < 0.001) was higher in girls than in boys (Figure 3B), while miR-143-5p did not differ significantly among the groups (1.000 ± 0.106 vs 0.958 ± 0.139; P = 0.290) (Figure 3B).

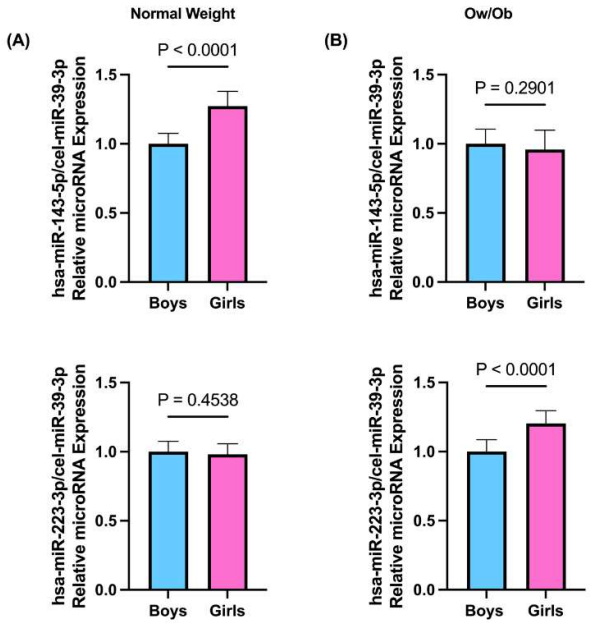


Figure 3. Plasma miRNA expression in boys and girls with NW and with Ow/Ob. (A) miR-143-5p expression and (B) miR-223-3p expression. miRNA expression was measured by RT-qPCR using cel-miR-39 as the reference for the $2^{-\Delta\Delta Ct}$ method. Differences were tested by an unpaired t-test. Results are presented as mean ± SD. NW: normal weight; Ow/Ob: Overweight/Obesity.

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Finally, we evaluated plasma miRNA expression according to IR status. Based on the established cutoff, 52.75% of the children were classified as having IR. As shown in Figure 4A, children with IR showed higher miR-143-5p expression levels than those without IR (1.000 ± 0.180 vs 4.371 ± 0.153; P < 0.001). Similarly, miR-223-3p expression levels were higher in children with IR (1.000 ± 0.118 vs 1.329 ± 0.111; P < 0.001) (Figure 4B)

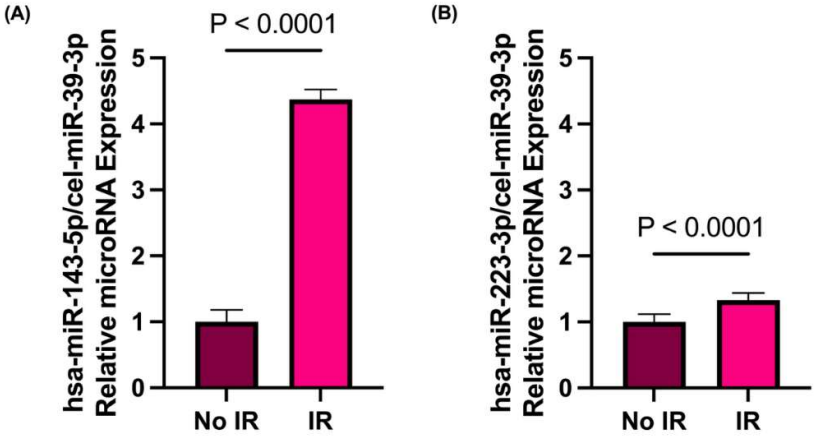


Figure 4. The plasma miRNA expression in children with and without insulin resistance. (A) miR-143-5p expression and (B) miR-223-3p expression. miRNA expression was measured by RT-qPCR using cel-miR-39 as a reference for the 2-ΔΔCt method. Differences were tested by an unpaired t-test. Results are presented as mean ± SD. IR: Insulin resistance.

Associations Between Plasma miR-143-5p and miR-223-3p Expression and Cardiometabolic Risk Parameters

Exploratory Spearman's correlations between plasma miR-143-5p and miR-223-3p expression and clinical and cardiometabolic risk parameters are presented in Supplementary Table S1. In these analyses, miR-143-5p expression was negatively correlated with BMI, BMI Z-score, waist circumference, and hip circumference (P < 0.05), and positively correlated with HDL-C levels (P < 0.05). Similarly, miR-223-3p expression was negatively correlated with hip circumference and positively correlated with added sugar intake (P < 0.05) (Supplementary Table S1).

However, as shown in Table 2, no significant associations were found between miR-143-5p or miR-223-3p expression and the evaluated cardiometabolic parameters (P > 0.05).

Table 2. Association of miRNA expression and cardiometabolic risk parameters.

	miR-143-5p			miR-223-3p		
	β	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**
CHO (mg/dL)	0.002	-0.004, 0.008	0.470	0.000	-0.006, 0.006	0.923
HDL-C (mg/dL)	0.009	-0.015, 0.034	0.439	-0.000	-0.025, 0.023	0.958
LDL-C (mg/dL)	-0.001	- 0.007, 0.005	0.709	-0.001	-0.008, 0.004	0.564
TG (mg/dL)	0.002	- 0.000, 0.004	0.089	0.001	-0.000, 0.004	0.194
Glucose (mg/dL)	0.010	- 0.009, 0.029	0.302	0.002	-0.017, 0.022	0.792
Insulin (µU/mL)	0.007	- 0.005, 0.020	0.271	0.010	-0.003, 0.023	0.146
HOMA-IR	0.035	- 0.022, 0.093	0.226	0.044	-0.015, 0.104	0.141

BMI: Body mass index; CHO: Cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides; HOMA-IR: Homeostatic model assessment of insulin resistance. β values represent unstandardized coefficients derived from linear regression models with miRNA expression as

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dependent variables, adjusted for sex, age, BMI and total energy intake. P values < 0.05 were considered statistically significant.

Associations Between Added Sugar Intake and miRNA expression and Cardiometabolic Risk Parameters

Exploratory Spearman's correlations between added sugar intake and cardiometabolic risk parameters are shown in Supplementary Table S2. According to these analyses, added sugar intake was positively correlated with miR-223-3p expression (P = 0.009) and glucose levels (P = 0.022). In the adjusted models, added sugar intake was positively associated with miR-223-3p expression (β = 0.188; 95% CI: 0.022 – 0.354; P = 0.027) and with glucose levels (β= 1.944; 95% CI: 0.133 – 3.755; P= 0.036) (Table 3). No significant associations were found for the remaining variables.

Table 3. Association of the added sugar intake with miRNA expression and cardiometabolic risk parameters.

	Added sugar (g)		
	β	**95% CI**	**P-value**
miR-143-5p	0.109	-0.056, 0.275	0.194
miR-223-3p	0.188	0.022, 0.354	0.027
CHO (mg/dL)	-3.125	-8.783, 2.533	0.275
HDL-C (mg/dL)	0.516	-0.991, 2.025	0.497
LDL-C (mg/dL)	-4.235	-9.796, 1.325	0.134
TG (mg/dL)	2.966	-12.038, 17.971	0.695
Glucose (mg/dL)	1.944	0.133, 3.755	0.036
Insulin (µU/mL)	1.481	-1.172, 4.135	0.270
HOMA-IR	0.443	-0.158, 1.045	0.146

BMI: Body mass index; CHO: Cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides; HOMA-IR: Homeostatic model assessment of IR. β values represent unstandardized coefficients derived from linear regression models with added sugar intake as the independent variable, adjusted for sex, age, BMI, and total energy intake. Results are presented as β and 95% CI. P values < 0.05 were considered statistically significant.

4. Discussion

The findings of this study suggest that added sugar intake is associated with alterations in circulating miRNA expression in children. Specifically, children with high added sugar intake exhibited lower plasma miR-143-5p expression and higher miR-223-3p expression compared with those with lower intakes. In addition, both miR-143-5p and miR-223-3p expression levels were higher in children with overweight/obesity and in those with insulin resistance. However, after adjustment, only added sugar intake remained positively associated with miR-223-3p expression and fasting glucose concentrations, whereas neither miRNA was associated with cardiometabolic risk parameters. Together, these findings suggest that added sugar intake may be related to specific circulating miRNAs involved in metabolic regulation, highlighting potential epigenetic pathways through which added sugars may affect metabolic health.

Childhood obesity is an emerging global health concern, as overweight and obesity during childhood have been shown to persist into adulthood [1], and higher added sugar intake has been associated with weight gain and an increased risk of obesity in children [5,6]. miRNAs are important regulators of metabolic balance [28]. Among these miRNAs, miR-143-5p and miR-223-3p have been implicated in metabolic regulation. miR-143 is conserved between humans and rats, and several studies have shown its important role in lipid metabolism, adipogenesis, and IR [13]. miR-223-3p has also been implicated in metabolic regulation and IR [29]. In our study, children with normal weight and high added sugar intake showed higher plasma miR-143-5p expression, whereas lower

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expression was observed in children with overweight/obesity. In the case of miR-223-3p, children with normal weight and overweight/obesity and high added sugar intake showed higher plasma miR-223-3p expression. A previous study showed that high-fructose intake increased miR-143-5p expression and reduced miR-223-3p expression in extracellular vesicles from plasma in murine models [19]. Conversely, high sucrose intake did not alter plasma levels [18].

After adjusting for sex, age, BMI, and total energy intake, added sugar intake in our study was positively associated with plasma miR-223-3p expression levels in children. Specifically, each 25 g/day increase in added sugar intake was associated with a 0.188-unit increase in plasma miR-223-3p expression, corresponding to an approximately 18.8% relative increase and suggesting a modest but potentially biologically relevant effect. In addition, each 25 g/day increase in added sugar intake was associated with an average 1.944 mg/dL increase in fasting glucose concentrations, supporting the relationship between higher added sugar consumption and early metabolic alterations in children. Taken together, these findings suggest a potential relationship between chronic added sugar intake and circulating miRNA expression. These results suggest that miR-223-3p may be particularly responsive to dietary sugars.

Additionally, plasma miR-143-5p expression was upregulated in children with overweight/obesity. This miRNA was negatively correlated with BMI, waist circumference, hip circumference and positively correlated with HDL-C levels. Similar results were found for serum miR-143-5p in children, adolescents, and adults with obesity [30,31]. In experimental models of high-fat diet-induced obesity, miR-143-5p expression was associated with body weight gain, mesenteric adipose tissue gain, and key adipogenesis markers [32]. Moreover, miR-143-5p promotes adipogenesis through the MAP2K5-ERK5 regulatory pathway [33]. Its inhibition decreases the expression of adipocyte-specific genes, such as PPAR-γ, aP2, and GLUT-4, as well as triglyceride levels. However, these associations were not maintained after adjustment.

miR-223-3p plasma expression was upregulated in children with overweight/obesity and negatively correlated with hip circumference. A study reported that plasma miR-223-3p expression was upregulated in children and adolescents with obesity and was inversely associated with obesity [34], suggesting that higher levels of this miRNA may be related to a lower probability of obesity. However, opposite results were observed in adults with obesity [31] and in patients with T2D [35]. In addition, miR-223-3p expression was upregulated in human omental adipose tissue from patients with obesity [36]. Functionally, miR-223-3p suppresses the pro-inflammatory activation of macrophages [37] and regulates NLRP3 and IL-1β production in mouse adipose tissue [38]. In vitro studies showed that TNF-α-stimulated preadipocytes, mimicking the inflammatory environment present in obesity, exhibited reduced secretion of this miRNA and increased intracellular accumulation, impairing glucose and lipid metabolism [35]. Moreover, studies have demonstrated that miR-223-3p promotes adipogenesis in mesenchymal stem cells [39] and human adipocytes [40]. Therefore, the altered expression of miR-143-5p and miR-223-3p in children with overweight/obesity may reflect adipose tissue dysfunction. However, neither miRNA was associated with cardiometabolic risk parameters in adjusted analyses.

No overall differences in plasma miR-143-5p and miR-223-3p expression were observed between boys and girls. However, when children were stratified by nutritional status, plasma miR-143-5p expression was upregulated in girls with normal weight, consistent with previous findings reported in boys and girls with normal weight [41]. Although the underlying mechanisms are not fully understood, this finding may be related to sex-specific differences in hormonal regulation, body composition, and pubertal development, which could modulate circulating miRNA profiles. Moreover, plasma miR-223-3p expression was upregulated in girls with overweight/obesity. Although sex-specific evidence for miR-223-3p in children is limited, altered circulating miR-223-3p levels have been reported in pediatric obesity [34], and sex-related differences in miRNA profiles have been described in adolescents with obesity [42]. Therefore, these findings suggest that the influence of sex on circulating miRNA expression may depend on nutritional and metabolic status. Nevertheless, these results should be interpreted cautiously, as sex-related differences in circulating

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miRNAs appear to be miRNA-specific and may vary according to population characteristics, age, metabolic status, and the biological matrix analyzed [43,44].

On the other hand, our study showed higher plasma miR-143-5p expression in children with IR. Previous in vitro studies demonstrated that FFAs, leptin, and resistin, key components of the obesogenic inflammatory microenvironment involved in IR, inhibited miR-143 expression in human adipocytes [45]. In addition, bone marrow macrophage-derived exosomal miR-143-5p has been shown to induce IR in hepatocytes by repressing MKP5 [46]. In our study, the expression levels of miR-223-3p were increased in children with IR. Similar findings have been reported in serum from adolescents with obesity and IR [47]. In contrast, a study conducted in adults with IR found that plasma miR-223-3p expression levels were lower and negatively correlated with the adipose tissue IR index [35]. In vitro studies have shown that the upregulation of miR-223-3p leads to the downregulation of GLUT4 in human adipocytes [48]. Moreover, miR-223-3p reduced the expression levels of NLRP3 mRNA, Bax and NLRP3 protein, thereby inhibiting apoptosis in endothelial cells [49]. Although both miR-143-5p and miR-223-3p were expressed at higher levels in children with IR, neither miRNA was associated with insulin, HOMA-IR, or glucose concentrations after adjustment for age, sex, BMI, and total energy intake. Therefore, these findings should be interpreted cautiously and may indicate that the roles of miR-143-5p and miR-223-3p in obesity-related IR are complex and may vary across tissues, cell types, and biological compartments analyzed.

Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

5. Conclusions

High added sugar intake was associated with altered plasma miRNA expression in children, characterized by lower miR-143-5p expression and higher miR-223-3p expression. In addition, both miR-143-5p and miR-223-3p expression levels were higher in children with overweight/obesity and in those with insulin resistance. After adjustment, added sugar intake was positively associated with miR-223-3p expression and fasting glucose concentrations, whereas neither miRNA was associated with cardiometabolic risk parameters. These findings suggest that added sugar intake may be related to specific circulating miRNAs involved in metabolic regulation, highlighting potential epigenetic pathways and early molecular alterations through which added sugars may affect metabolic health.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request due to ethical restrictions and the need to protect participant confidentiality in accordance with institutional guidelines.

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Abbreviations

The following abbreviations are used in this manuscript:

3′UTR	3′ untranslated region
BMI	Body mass index
BMI Z-score	Body mass index Z-score
cDNA	Complementary DNA
CHO	Cholesterol
ENSANUT	National Health and Nutrition Survey
FFAs	Free fatty acids
FFQ	Food frequency questionnaire
GLUT-4	Glucose transporter type 4
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment of insulin resistance
IL-1β	Interleukin-1 beta
IR	Insulin resistance
LDL-C	Low-density lipoprotein cholesterol
MAP2K5-ERK5	Mitogen-activated protein kinase kinase 5–extracellular signal-regulated kinase 5
miR-143-5p	microRNA-143-5p
miR-223-3p	microRNA-223-3p
miRNA	microRNA
mRNA	Messenger RNA
NLRP3	NOD-like receptor family pyrin domain containing 3
NW	Normal weight
Ow/Ob	Overweight/obesity
PPAR-γ	Peroxisome proliferator-activated receptor gamma
RNA	Ribonucleic acid
T2D	Type 2 diabetes
TG	Triglycerides
TNF-α	Tumor necrosis factor alpha
WHO	World Health Organization

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