Article

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Altered Expression of the MEG3–FTO–ATF4 Axis and Lipid Metabolism Genes in PBMCs from Children with Obesity and Its Associations and Added Sugar Intake

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Abstract

Background: Obesity and its complications have increased in both adults and children, with pediatric populations developing metabolic disorders earlier ages. Long non-coding RNAs, particularly *MEG3*, are involved in obesity through regulation of lipid metabolism genes including *ATF4*, *FTO*, *SREBP1*, *FASN*, and *ACACA*. However, data on MEG3 expression in pediatric obesity are limited. This study evaluated *MEG3*, *FTO*, and *ATF4* expression in PBMCs from children with obesity and their associations with added sugar intake and lipid metabolism genes. **Methods**: In this cross-sectional study a total of 71 children aged of 6 to 12 years were included (28 normal weight and 43 with obesity). Anthropometrical and clinical parameters and dietary added sugar consumption were analyzed. Real-time PCR was performed to assess *MEG3*, *FTO*, *ATF4*, *SREBP1*, *FASN* and *ACACA* gene expression in peripheral blood mononuclear cells. **Results**: The expression of *MEG3*, *ATF4*, *FTO*, *SREBP1*, *FASN*, and *ACACA* was decreased in children with obesity. MEG3 and FTO showed sex-dependent expression in children without obesity, while additional sex-related differences were observed for *SREBP1*, *FASN*, *ACACA*, *FTO*, and *MEG3* in children with obesity. *MEG3* was correlated with increased levels of *ATF4* and *SREBP1*, and moderately with *FTO* and *FASN*. In insulin resistance (IR) children, *MEG3*, *ATF4*, *FTO*, *ACACA*, and *SREBP1* were reduced, while *FASN* was increased. Added sugar intake negatively correlated with *FTO*, *SREBP1*, and *ACACA*. **Conclusions**: The *MEG3–FTO–ATF4* axis was altered in children with obesity, showing sex- and IR-related differences. Added sugar intake correlated negatively with lipid metabolism gene expression.

Keywords: LncRNA MEG3; Pediatric Obesity; Lipid Metabolism; Added Sugar Intake

1. Introduction

Obesity and its related diseases have increased in recent decades, both adults and children, becoming a major public health concern. Epidemiological studies have demonstrated a significant increase in the prevalence of overweight and obesity among children worldwide. For instance, the prevalence of obesity in children aged 5 – 14 years has tripled worldwide, increasing from 2.0% in 1990 to 6.8% in 2021, and it is estimated that by 2050, the prevalence will reach 15.6% [1]. In Mexico, the prevalence of obesity among children aged 5 – 11 years has been reported at 18.1% [2]. Obesity is the main risk factor for insulin resistance (IR), type 2 diabetes (T2D), dyslipidemia, and cardiovascular diseases. The pediatric population in Mexico is considered prone to developing these metabolic complications at earlier ages [3]. As for genetic factors, several genes involved in lipid metabolism have been identified as potential contributors to the development of obesity and its metabolic complications [4].

Long non-coding RNAs (lncRNAs), which are longer than 200 nucleotides, play a role in development of IR by regulating lipid and carbohydrate metabolisms and inflammation [5]. Maternally expressed gene 3 (MEG3), a prominent gene located on chromosome 14 in humans, encodes a lncRNA approximately 700 nucleotides [6]. It is highly conserved and enriched in several tissues, including peripheral blood mononuclear cells (PBMCs) [7–9]. MEG3 participates in the pathogenesis of obesity and obesity-related disease, including cardiovascular diseases and T2D [10–12], and its differential expression offers significant potential for its use as both biomarkers and therapeutic targets [5]. Although MEG3 expression has been studied mainly in adult populations, there is limited evidence in pediatric populations in this context.

In addition to genetic factors, dietary factors also play a key role in the development of obesity and its metabolic complications. High intake of added sugars has been associated with increased risk of obesity, IR, and dyslipidemia, especially in children [13,14]. Emerging evidence suggests that excessive consumption of added sugars may alter lipid metabolism and contribute to the dysregulation of gene expression, including lncRNAs, such as MEG3 [15].

Recent studies demonstrated that MEG3 modulates key lipid metabolism genes, such as ATF4, FTO, SREBP1c, FASN, and ACACA. For instance, ATF4 is a key transcription factor in the regulation of lipids biosynthesis [16], and its deficiency is involved in reduction of hepatic lipogenesis through the regulation of SREBP1c, PPARγ and FASN expression [17]. FTO increases lipid accumulation in hepatocytes by promoting the nuclear translocation and maturation of SREBP1c [18]. Given these findings, the evaluation of the expression of these genes in PBMCs allows for a minimally invasive approach to explore molecular alterations related to obesity and dietary factors in children. Accordingly, the present study aimed to evaluate the expression of MEG3, FTO, and ATF4 in PBMCs from children with obesity and to assess their associations with added sugar intake and the expression of lipid metabolism-related genes.

2. Materials and Methods

2.1. Study Participants

A total of 71 unrelated children between the ages of 6 to 12 were enrolled from Mexico City (28 with normal weight and 43 with obesity) to evaluate the association of the consumption of added sugar with gene expression related to lipid metabolism, obesity, and cardiometabolic risk factors. The research was approved by the ethics committee of the Instituto Mexicano del Seguro Social (R-2024-785-061) and was conducted in compliance with the Declaration of Helsinki. Assent was obtained from all participating children, and informed consent was provided by their parents or legal guardians. 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.

Weight and height were measured using a digital scale (Seca, Hamburg, Germany) and a portable stadiometer (Seca 225, Hamburg, Germany), respectively. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). BMI values were converted into age- and sex-specific percentiles using the growth charts provided by the Centers for Disease Control and Prevention (CDC). Based on the CDC 2000 growth reference, children were classified as having normal weight if their BMI was ≥5th and <85th percentile, and as having obesity if their BMI was ≥95th percentile.

2.2. Biochemical Measurements

Following an 8-hour overnight fast, venous blood samples were collected to assess biochemical parameters, including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (Total CHO), triglycerides (TG), glucose and insulin. Serum concentrations of LDL-C, HDL-C, Total CHO, TG, and glucose were determined 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). To determine the HOMA-IR, we multiply the fasting glucose concentration (mg/dL) by the fasting insulin concentration (µU/mL) and dividing by 405. IR was also evaluated in children, according to HOMA-IR values: those with HOMA-IR ≤ 3 were considered as not having IR, and those with HOMA-IR > 3 as having IR.

2.3. Dietary Added Sugar Consumption

Dietary added sugar consumption was assessed using a semi-quantitative food frequency questionnaire (FFQ) that inquiries about food intake over the past month [19]. It was designed using the Food Frequency Questionnaire from the Mexican National Health and Nutrition Survey (ENSANUT) for school-aged children (from the National Institute of Public Health) as a reference. Reference portions were estimated based on 24-hour dietary recalls from ENSANUT for school-aged children, and all portions were expressed in household measurements.

To estimate added sugar intake, a specific food composition database was developed to process the data collected through the questionnaire. This database was built using the Mexican Food Composition Table (BAM) [20]. The FFQ was analyzed following the same methodological criteria used in the analysis of grams and nutrients in the ENSANUT FFQ [21].

2.4. RT-qPCR

Total RNA was extracted from whole venous blood using the TRIzol reagent (Thermo Scientific, USA), according to the manufacturer's instructions. The concentration and purity of the extracted RNA were subsequently evaluated using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, USA). To remove genomic DNA, RNA was treated with DNase I (Thermo Fisher Scientific) according to the manufacturer's instructions. RNA integrity was assessed using agarose gel electrophoresis. cDNA was synthesized from 500 ng of total RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). The resulting cDNA was subsequently used as a template for RT-qPCR, which was conducted using a Maxima SYBR Green/ROX qPCR Master Mix Thermo Scientific, USA) on a 7900HT Fast Real-Time PCR System (Applied Biosystems, California, USA). To determine gene expression, the following primers were used: FTO (Assay ID: QT00088802), *ATF4* (Assay ID: QT00074466), *SREBP1* (Assay ID: QT00036897), *FASN* (Assay ID: QT00030618), *ACACA* (Assay ID: QT01670053), *RPLP0* (Assay ID: QT01839887), and *MEG3* (Assay ID: LPH02974A) (Qiagen; Hilden, Germany). mRNA relative concentrations were normalized with Ct values of *RPLP0*, and values were calculated using $2^{-\Delta\Delta Ct}$ formula.

2.5. Statistical Analysis

The normality of continuous variables was assessed using the Shapiro-Wilk test. Variables with normal distribution were analyzed using the student's t-test, while non-normally distributed variables were analyzed using the Mann-Whitney U test. Differences between categorical variables were evaluated using the Chi-square test. To evaluate the relationship between variables, Pearson's correlation was used for parametric data and Spearman's correlation for nonparametric data.

Due to the non-normal distribution of residuals in the linear regression model, quantile regression was used to evaluate associations across different points of the outcome distribution, adjusting for age, sex, and total energy intake. No variable standardization was applied. For all analyses, a two-tailed *p*-value of <.05 was statistically meaningful. All statistical analyses were performed using STATA (version 19.5; StataCorp LLC, USA).

3. Results

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3.1. Characteristics of the Study Population

The health outcome variables of the children are shown in Table 1. The mean age was 8.94 ± 1.68 years. Girls represented 60.56% of the sample, while boys accounted for 39.44%. BMI, insulin, HOMA-IR, and TG levels were significantly higher in the obesity group compared to the without obesity group. Moreover, HDL-C concentration was significantly lower in children with obesity than in those without obesity. No differences were found in glucose, total cholesterol, or LDL-C between the two groups. Finally, no significant differences in added sugar and total energy intake were observed between the groups.

3.2. Expression of Genes Involved in Lipid Metabolism in Children with Obesity

Results showed alterations in gene expression associated with lipid metabolism in children with obesity. As shown in Figure 1, the mRNA expression of *SREBP1 (p= 0.0176), FASN* (p= 0.0412), *ACACA* (p= 0.0255), *FTO* (p <0.0001), and *ATF4* (p <0.0001) were remarkably reduced in obesity group compared to control group. Moreover, the expression of lncRNA *MEG3* (p = 0.0197) was increased in children with obesity compared to those with normal weight. This finding suggests that obesity in children decrease the expression of gene involved with lipid metabolism.

In addition, gene expression was also analyzed according to sex in both the normal weight and obesity groups. As shown in Figure 2, among children with normal weight, the expression of *FASN* (p= 0.0293) was higher in boys compared to girls. In contrast, the expression of *ACACA* (p= 0.0309) and *MEG3* (p= 0.0144) was higher in girls compared to boys. However, no differences were found in the expression of *SREBP1*, *FTO*, or *ATF4* between those children. Instead, in children with obesity, the expression of *FASN* (p= 0.0316), *FTO* (p= 0.0443), and *MEG3* (p= 0.0487) was higher in boys compared to girls as shown in Figure 3. No differences were found in the expression of *SREBP1*, *ACACA*, or *ATF4* between boys and girls with obesity. Thus, evidence indicate *FASN*, *ACACA*, *FTO* and *MEG3* are differential expressed in girls and boys.

Table 1. Demographic, clinical and metabolic parameters.

Variable	Total (n= 71)	Without obesity (n= 27)	With obesity (n= 44)	P value
Age (y)	8.94 ± 1.680	9.111 ± 1.740	8.84 ± 1.660	0.514^{a}
Sex, n (%)				
Male	28 (39.44)	9 (32.14)	19 (67.86)	0.410^{b}
Female	43 (60.56)	18 (41.86)	25 (58.14)	
Waist circ (cm)	74.338 ± 13.462	60.833 ± 7.302	82.625 ± 8.831	< 0.001^{a}
Hip circ (cm)	82.047 ± 12.037	70.940 ± 6.962	88.863 ± 9.031	< 0.001^{a}
BMI (kg/cm^2)	22.725 (16.405—25.184)	16.113 (15.527—16.645)	24.292 (23.787—25.410)	< 0.001^{c}
BMI percentile	76.352 ± 30.283	41.555 ± 20.905	97.704 ± 1.373	< 0.001^{a}
Glucose (mg/dL)	87.288 ± 7.991	88.185 ± 6.314	86.738 ± 8.890	0.463^{a}
Insulin (µIU/ml)	14.430 (8.400—21.400)	8.400 (7.200—9.550)	17.880 (15.000—22.100)	< 0.001^{c}
HOMA-IR	3.066 (1.767—4.796)	1.794 (1.596—2.092)	3.726 (3.123—4.801)	< 0.001^{c}
≥3, n (%)	36 (50.70)	6 (22.22)	30 (68.18)	< 0.001^{b}
Total CHO (mg/dL)	164.253 ± 27.204	164.185 ± 31.027	164.295± 24.953	0.986^{a}
HDL-C (mg/dL)	47.983 ± 10.577	52.981 ± 8.716	44.915 ± 10.529	< 0.001^{a}
LDL-C (mg/dL)	85.982 ± 25.146	86.937 ± 28.916	85.396 ± 22.866	0.804^{a}
TG (mg/dL)	129.7 (89.100—219.300)	100.600 (85.345—137.136)	157.050 (123.967—200.893)	0.009^{c}
Added sugar (g)	73.724 ± 36.195	71.751 ± 44.585	74.934 ± 30.710	0.766^{a}
≥ 50 g, n (%)	58 (81.69)	20 (74.07)	38 (86.36)	0.194^{b}

Total energy intake (Kcal)	2383.5 (1983—2787)	2340 (1908—2698)	2490 (2085—2870)	0.294[c]

BMI: Body mass index; HOMA-IR: Homeostatic model assessment of insulin resistance; CHO: Cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides. Data are presented as the mean ± standard deviation (SD) or the median (interquartile range) for parametric and nonparametric data, respectively. [a]Student's t test; [b]Chi-square test; [c]Mann-Whitney test.

Based on the IR criterion, 50.70% of children were diagnosed with IR. As show in the Figure 4, the expression of *SREBP1* ($p = 0.028$), *ACACA* ($p = 0.038$), *FTO* ($p = 0.045$), *ATF4* ($p = 0.041$) and *MEG3* ($p = 0.021$) was reduced in children with IR compared to those without IR. These results suggest the downregulation of these genes may be involved in IR.

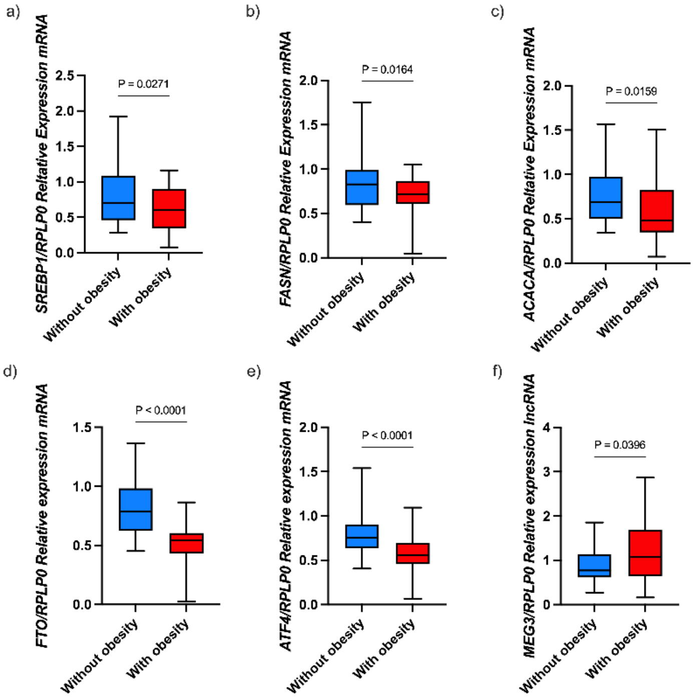

Figure 1. The expression of lipid metabolism genes in children with and without obesity. Gene expression of a) *SREBP1*, b) *FASN*, c) *ACACA*, e) *FTO*, e) *ATF4*, and f) *MEG3* expression was determined by RT-qPCR using *RPLP0* as reference for the $2^{-\Delta\Delta Ct}$ method. Differences were tested by the Mann-Whitney test. Data are presented as means ± SD.

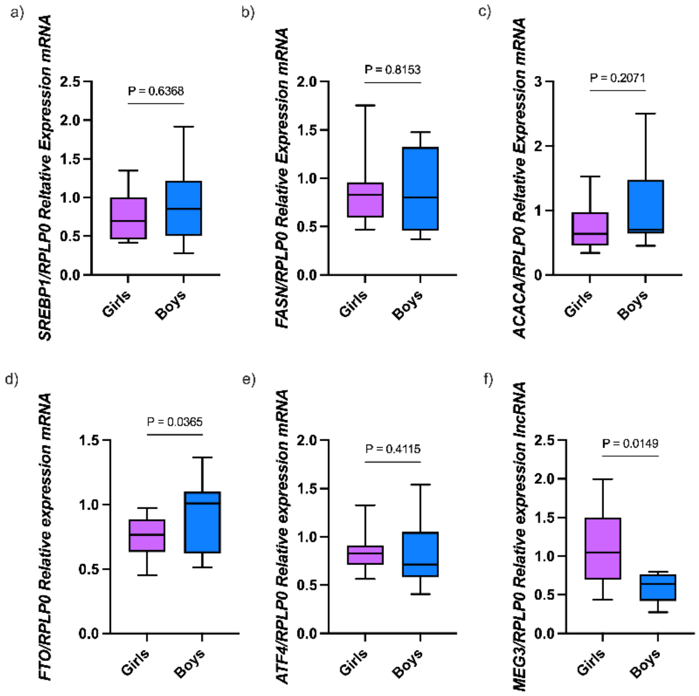

Figure 2. The expression of lipid metabolism genes in girls and boys without obesity. Gene expression of a) *SREBP1*, b) *FASN*, c) *ACACA*, e) *FTO*, e) *ATF4*, and f) *MEG3*. mRNA expression was determined by RT-qPCR using *RPLP0* as reference for the $2^{-\Delta\Delta Ct}$ method. Differences were tested by the Mann-Whitney test. Data are presented as means ± SD.

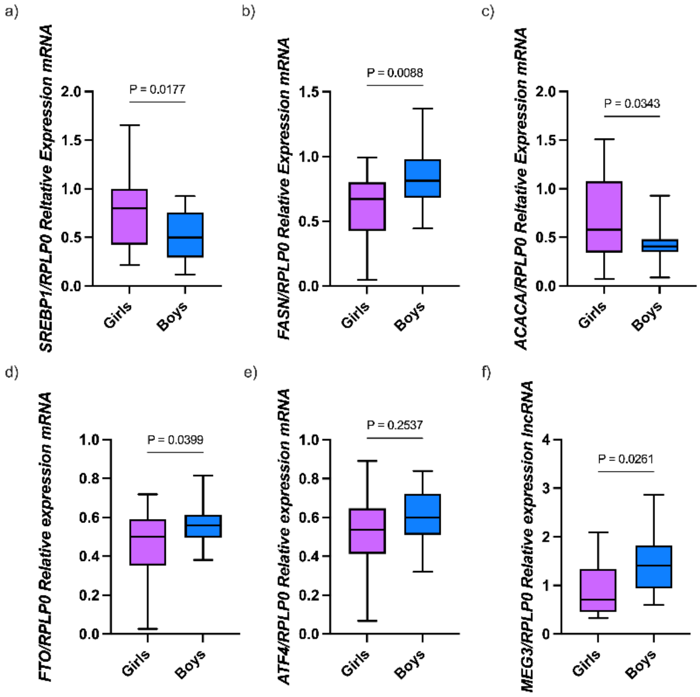

Figure 3. The expression of lipid metabolism genes in girls and boys with obesity. Gene expression of a) *SREBP1*, b) *FASN*, c) *ACACA*, e) *FTO*, e) *ATF4*, and f) *MEG3*. mRNA expression was determined by RT-qPCR using *RPLP0* as reference for the $2^{-\Delta\Delta Ct}$ method. Differences were tested by the Mann-Whitney test. Data are presented as means ± SD.

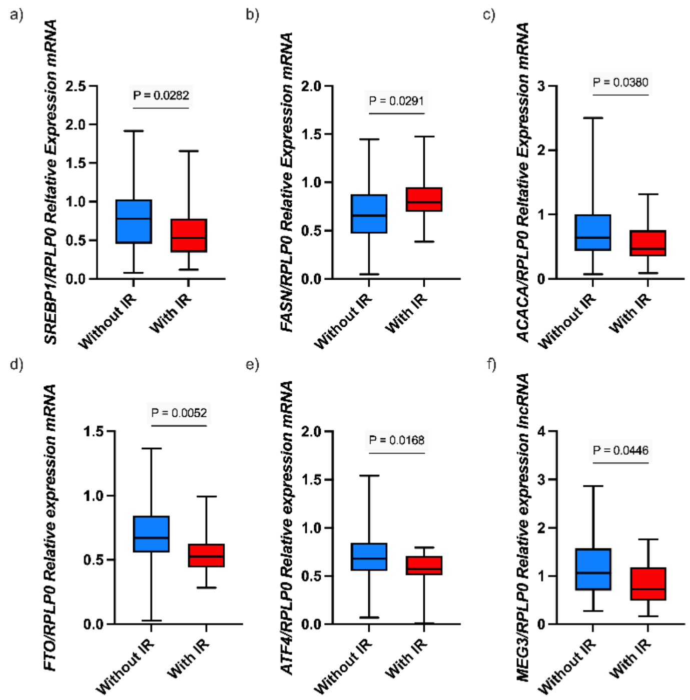

Figure 4. The expression of lipid metabolism genes in children with and without IR. Gene expression of a) *SREBP1* b) *FASN* c) *ACACA* e) *FTO* and f) *ATF4*. mRNA expression was determined by RT-qPCR using *RPLP0* as reference for the $2^{-\Delta\Delta Ct}$ method. Differences were tested by the Mann-Whitney test. Data are presented as means ± SD.

3.2. Associations Between lncRNA MEG3 and Gene Expression and Biochemical Parameters

The correlation between the expression of lncRNA *MEG3* and of genes involved with lipid metabolisms was analyzed and the results are presented in Table 2. A positive correlation was found between the expression of *MEG3* and the expression of *SREBP1* (Rho= 0.408; p= 0.001), *FTO* (Rho= 0.389; p = 0.001) and *ATF4* (Rho= 0.422; p < 0.001), whereas it showed a positive correlation with *FASN* (Rho= 0.238; p= 0.025). Quantile regression analysis was performed to examine the association between the expression levels of lncRNA *MEG3* and genes involved in lipid metabolism. All models were adjusted for sex, age and BMI. β coefficients and p-values are presented in Supplementary Table 1. In quantile regression analysis, the expression of *MEG3* was positively associated with *SREBP1* (β= 0.322; p < 0.001), *FASN* (β= 0.186; p= 0.001), ACACA (β= 0.174; p= 0.033), *FTO* (β= 0.147; p= 0.003) and *ATF4* (β=0.116; p= 0.007) expression. The above suggest that *MEG3* expression may be modulating the expression of *SREBP1*, *FASN*, *FTO* and *ATF4*.

Table 2. Spearman's correlation coefficients between MEG3 and lipid metabolism genes.

Gene	Rho	P Value
SREBP1	**0.408**	**0.001**
FASN	**0.238**	**0.025**
ACACA	0.130	0.307
FTO	**0.389**	**0.001**
ATF4	**0.422**	**< 0.001**

SREBP1: Sterol Regulatory Element-Binding Protein 1; FASN: Fatty Acid Synthase; ACACA: Acetyl-CoA Carboxylase Alpha; FTO: Fat Mass and Obesity-Associated Gene; ATF4: Activating Transcription Factor 4. Rho values correspond to the Spearman correlation coefficients. P values < 0.05 were considered statistically significant.

The correlation of the expression of the gene involved with lipid metabolism and lncRNA *MEG3* with clinical and metabolic parameters was analyzed and the results are presented in Table 3. There was a negative correlation between *SREBP1* and glucose (Rho= -0.260; p= 0.029), insulin (Rho= -0.354; p= 0.003), and HOMA-IR (Rho= -0.373; p= 0.001). Likewise, *FASN* showed a positive correlation between glucose (Rho= 0.304; p= 0.012), and HOMA-IR (r= 0.254; p= 0.040). In the case of *ACACA*, there was a negative correlation with BMI (Rho= -0.347; p= 0.003), waist circumference (Rho= -0.408; p <0.001), hip circumference (Rho= -0.307, p= 0.009), insulin (Rho= -0.425; p < 0.001), HOMA-IR (Rho= -0.438; p < 0.001), and triglycerides (Rho= -0.276; p= 0.019), whereas it showed a positive correlation with HDL-C (Rho= 0.302; p= 0.010). Additionally, *FTO* showed a negative correlation between BMI percentile (Rho= -0.452; p < 0.001), waist circumference (Rho= -0.497; p <0.001), hip circumference (Rho= -0.466; p < 0.001), insulin (Rho= -0.426; p < 0.001), and HOMA-IR (Rho= -0.419; p < 0.001). Similarly, *ATF4* showed a negative correlation between BMI percentile (Rho= -0.381; p <0.001), waist circumference (Rho= -0.340; p= 0.003), hip circumference (Rho= -0.293; p= 0.012), insulin (Rho= -0.267; p= 0.026) and HOMA-IR (Rho= -0.241; p= 0.046). However, *MEG3* did not show a significant correlation with any variable.

Table 3. Spearman's correlation coefficients between lncRNA *MEG3*, lipid metabolism genes and clinical and biochemical parameters.

Variable	***SREBP1c***		***FASN***		***ACACA***		***FTO***		***ATF4***		***MEG3***	
	Rho	**P-value**	**Rho**	**P-value**	**Rho**	**P-value**	**Rho**	**P-value**	**Rho**	**P-value**	**Rho**	**P-value**
BMI percentile	-0.194	0.107	-0.084	0.469	-0.347	0.003	-0.452	< 0.001	-0.383	< 0.001	0.069	0.585
Waist circ (cm)	-0.223	0.062	-0.023	0.852	-0.408	< 0.001	-0.497	< 0.001	-0.340	0.003	0.029	0.816
Hip circ (cm)	-0.185	0.124	-0.002	0.981	-0.307	0.009	-0.466	< 0.001	-0.293	0.012	0.020	0.874
Glucose (mg/dL)	-0.260	0.029	0.304	0.012	-0.187	0.117	-0.074	0.536	0.137	0.253	-0.006	0.956
Insulin (µIU/ml)	-0.354	0.003	0.223	0.073	-0.425	< 0.001	-0.426	< 0.001	-0.267	0.026	-0.168	0.193
HOMA-IR	-0.373	0.001	0.254	0.040	-0.438	< 0.001	-0.419	< 0.001	-0.241	0.046	-0.193	0.135
Total CHO (mg/dL)	-0.071	0.558	-0.082	0.507	0.105	0.381	-0.064	0.592	-0.078	0.517	0.110	0.289
HDL-C (mg/dL)	0.046	0.701	-0.168	0.172	0.302	0.010	0.202	0.090	0.127	0.288	-0.066	0.606
LDL-C (mg/dL)	-0.042	0.725	-0.024	0.843	0.163	0.174	-0.010	0.929	-0.119	0.320	0.090	0.479
TG (mg/dL)	-0.039	0.743	0.090	0.465	-0.276	0.019	-0.209	0.079	-0.077	0.518	0.036	0.774

BMI: Body Mass Index; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; CHO: Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; LDL: Low-Density Lipoprotein Cholesterol; TG: Triglycerides; SREBP1: Sterol Regulatory Element-Binding Protein 1; FASN: Fatty

Acid Synthase; ACACA: Acetyl-CoA Carboxylase Alpha; FTO: Fat Mass and Obesity-Associated Gene; ATF4: Activating Transcription Factor 4; MEG3: Maternally Expressed Gene 3. Rho values correspond to the Spearman correlation coefficients. P values < 0.05 were considered statistically significant.

Quantile regression analysis was performed to examine the association between the expression levels of genes involved in lipid metabolism and clinical and biochemical parameters. All models were adjusted for sex, age and BMI. β coefficients and p-values are presented in Supplementary Table 2. In quantile regression analysis, the expression of *SREBP1* was negatively associated with glucose ($p < 0.05$), and HOMA-IR ($p < 0.05$). However, *FASN, ACACA, FTO, ATF4* and *MEG3* did not show a significant association with any variable ($p > 0.05$).

3.3. Association Between Added Sugar Intake and Molecular and Biochemical Parameters

Although no significant differences in added sugar intake were observed between children without obesity and those with obesity (Table 1), analyses revealed significant correlations with gene expression as show in Table 4. There was a significant negative correlation between added sugar intake and *SREBP1* (Rho= -0.317; p= 0.026), *ACACA* (Rho= -0.314; p= 0.026), and *FTO* (Rho= -0.355; p= 0.011), whereas *FASN*, *ATF4* and *MEG3* no significant correlation were observed.

Table 4. Spearman's correlation coefficients between added sugar intake and lipid metabolism genes.

Gene	*Rho*	P-value
SREBP1	**-0.317**	**0.026**
FASN	-0.003	0.979
ACACA	**-0.314**	**0.026**
FTO	**-0.355**	**0.011**
ATF4	-0.113	0.451
MEG3	-0.084	0.599

SREBP1: Sterol Regulatory Element-Binding Protein 1; FASN: Fatty Acid Synthase; ACACA: Acetyl-CoA Carboxylase Alpha; FTO: Fat Mass and Obesity-Associated Gene; ATF4: Activating Transcription Factor 4; MEG3: Maternally Expressed Gene 3. Rho values correspond to the Spearman correlation coefficients. P values < 0.05 were considered statistically significant.

Quantile regression analysis was performed to examine the association between the added sugar intake and expression levels of *MEG3* and genes involved in lipid metabolism. All models were adjusted for sex and total energy intake. β coefficients and p-values are presented in Supplementary Table 3. In quantile regression analysis, the added sugar intake was significant negative associated with *SREBP1* (β= -0.360; = 0.050) and *FTO* (β= -0.221: p= 0.032) expression, whereas *FASN*, *ACACA*, *ATF4* and *MEG3* no significant association were observed.

4. Discussion

Emerging studies in both animal models and humans have begun to elucidate the molecular mechanisms of the lncRNA *MEG3* in obesity and obesity-related diseases [22]. However, its clinical relevance in the context of pediatric obesity remains poorly understood.

In this study, the expression of the *MEG3*, known to be involved in lipid metabolism, was evaluated in PBMCs from children with obesity, and compared with children with normal weight. Our results show an elevated expression of *MEG3* in children with obesity. Consistent with recent findings reporting elevated expression of *MEG3* in the serum of children with obesity [7]. Interestingly, we observed a sex-dependent expression pattern: in children from the control group, *MEG3* expression was higher in girls than in boys, whereas in the obesity group, *MEG3* expression were higher in boys than in girls.

Previous studies have reported positive correlation between *MEG3* and lipid metabolism genes, such as *FASN* and *PPARG*, in the subcutaneous adipose tissue of females with obesity [23]. In line with these observations, our data showed that *MEG3* expression in PBMCs was associated with the expression of *SREBP1, FASN, FTO*, and *ATF4* in children with obesity, but no significant correlations were found with anthropometric and biochemical parameters.

Experimental studies *in vivo* and *in vitro* models have demonstrated that *Meg3* inhibits of de novo lipogenesis by reducing the expression of *Acc* and *Fasn* [10,11]. Furthermore, silencing *Meg3* in mice feed high fat diet led to reduce body weight, lower glucose and insulin levels, decreased inflammation and diminished fat accumulation [12].

Our results showed that children with IR exhibited lower of *MEG3* expression. This finding contrasts with increased *MEG3* levels in adult patients with T2D [8,9]. In animal studies, *Meg3* upregulating worsen hepatic IR by increasing *Foxo1* expression [24]. Additionally, *Meg3* can act as a competing endogenous RNA, modulating miR-214 and consequently inducing *Atf4* expression, thereby contributing to hepatic IR [25]. Therefore, the evidence suggests that the reduction expression of *MEG3* could be related to the novo lipogenesis present in children with obesity and IR. However, it is needing more experimental studies in humans for elucidate the function of *MEG3* in this context.

Regarding *ATF4* gene, in our results found a decreased expression in PBMCs from children with obesity compared to those without obesity. In contrast, a previous study reported no changes in *ATF4* expression in women with obesity compared to those with normal weight [26]. Also, that study showed a positive correlation between *ATF4* expression and BMI, cholesterol, triglycerides, HDL-C [26]. In contrast to these observations, our data revealed no significant correlations between *ATF4* expression and these lipid parameters.

ATF4 is a key transcription factor in regulating of lipids biosynthesis [16]. Previous experimental studies conducted in *Atf4*-deficient animal models demonstrated a diminished hepatic lipogenesis induced by high fructose intake, due to reduced expression of, *Srebp1c, Pparγ* and *Fasn* expression [17]. *In vitro* experiments reported that *Atf4* inhibits the transcription of *Srebp1*, a central regulator of fatty acid synthesis [27]. In our study, *ATF4*, *SREBP1* and *FASN* expression was reduced in PBMCs from children with obesity, contrasting with reports showing no differences in women with obesity [28]. Interestingly, we observed a significant positive correlation between *ATF4* and *SREBP1, FASN* and *ACACA* expression in PBMCs from children with obesity (Supplementary Table 4). Moreover, *SREBP1* and *FASN* expression correlated with glucose homeostasis markers, whereas *ACACA* expression correlated with glucose homeostasis markers, clinical and lipid parameters. After adjusted for sex and age, *ATF4* expression was negatively associated with clinical parameters, while *SREPB1* expression was negatively associated with glucose homeostasis markers (Supplementary Table 2). These findings suggest that the *ATF4–SREBP1* interaction may be regulated differently in pediatric obesity, possibly reflecting early compensatory mechanisms.

On the other hand, we reported that children with IR presented a decreased in *ATF4* expression compared to those without IR. Conversely, recent studies have reported high *ATF4* expression in PBMCs from adult patients with T2D compared to non-diabetic subjects [29,30]. That study also found a positive correlation between *ATF4* expression and *MEG3* expression [29]. Consistently, we also observed a significant correlation between *ATF4* and *MEG3* expression, in PBMCs from children [29]. These results suggest that *MEG3* may regulate *ATF4* expression through the mechanisms molecular mentioned above. Interestingly, in contrast to adult data [29], we found negative correlations between *ATF4* expression and insulin levels, HOMA-IR values, and BMI in the pediatric population.

In experimental studies have reported that, the inhibition of *Atf4* reversed hepatic IR induced by acute brain endoplasmic reticulum stress [31]. Conversely, a recent study in β-cell–specific *Atf4*-deficient animal models exhibited exacerbated diabetes, shown by hyperglycemia, as well as markers of dedifferentiation [32,33]. Furthermore, *Atf4* in β-cells is required for glucose homeostasis during aging and metabolic stress, but not in young mice [33]. Interestingly, recent *in vitro* experiments have also suggested a regulatory interplay between *Fto* and *Atf4*, showing that *Fto* expression may promote gluconeogenesis by inhibiting *Atf4* expression [34]. Therefore, the results show an opposing trend found between children and adults, which may reflect developmental or compensatory differences in the regulation of *ATF4*. This discrepancy may suggest that, in early stages of metabolic dysfunction, reduced *ATF4* expression represents an adaptive response aimed at mitigating cellular stress or limiting further metabolic impairment.

According to *FTO* gene**,** a previous study demonstrated that high *FTO* expression in PBMCs is associated with increased body mass [35]. In contrast, our results showed decreased *FTO* expression levels in PBMCs from children with obesity, with a significant negative correlation observed with BMI, and waist and hip circumference. Conversely, in adolescent and adult patients with overweight and obesity have reported no significant changes in *FTO* expression in PBMCs and adipose tissue compared to normal weight subjects [36–39]. These findings suggest that the reduce *FTO* expression observed in children may reflect age-specific regulatory mechanisms of *FTO* in early stages of metabolic dysregulation.

In addition, experimental studies have shown that *Fto* increases lipid accumulation in hepatocytes by promoting the nuclear translocation and maturation of Srebp1 [18]. Consistent with these findings, our results showed a strong positive correlation between *FTO* and *SREBP1, FASN* and *ACACA* in PBMCs (Supplementary Table 4). However, after adjusting for sex and age, *FTO* expression was negatively associated with anthropometric parameters (Supplementary Table 2).

On the other hand, recent experimental studies in animal models have demonstrated that m6A modification of *Meg3* by demethylase activity of *Fto* reduces *Meg3* expression [40]. Conversely, our study found a positive correlation between *FTO* and *MEG3* expression in PBMCs from children with obesity. These findings suggest that the regulation of *MEG3* expression by *FTO* may differ in humans. However, further studies are needed to elucidate the role of FTO activity in regulating *MEG3* expression in this context.

A previous study reported increased mRNA and protein expression of *FTO* in subcutaneous adipose tissue from women with higher HOMA-IR values. In that study, a positive correlation was also found between *FTO* expression and HOMA-IR index [41]. Conversely, our findings showed reduced *FTO* expression in PBMCs from children with IR compared to those without IR. Additionally, we found a strong negative correlation between *FTO* expression and both insulin levels and HOMA-IR values. In line with these observations, a recent study reported downregulation of *FTO* expression in human pancreatic islets from patients with diabetes [42]. That study also demonstrated that reduced *Fto* expression impaired insulin release in rat pancreatic cells [42]. Therefore, these findings suggest that *FTO* may play an important role in the development of T2D. However, further experimental and clinical studies are needed to elucidate the role in both children and adult in this context.

In addition to these molecular associations, we evaluated the potential influence of dietary added sugar intake on gene expression. Our analysis showed significant negative correlations between added sugar intake and the expression of *SREBP1*, *ACACA*, and *FTO* in PBMCs from children with obesity. Similar findings have been reported in adipose tissue, where a significant negative correlation was observed between *FTO* expression in adipose tissue and total carbohydrate intake in subjects with and without obesity [39]. Furthermore, after adjusting for sex and total energy intake, dietary intake of added sugar was negatively associated with the *FTO* and *SREBP1* expression in PBMCs from children with obesity. Specifically, intake > 50g of added sugar was associated with reductions of 0.221 and 0.360 units in of *FTO* and *SREPB1* expression levels in PBMCs, respectively (Supplementary table 3). These findings suggest that excessive added sugar intake may contribute to early dysregulation of lipid metabolism pathways mediated by FTO and SREBP1 in pediatric obesity.

The main limitations of this study are that gene expression was analyzed in PBMCs, which may not fully reflect expression patterns in primary metabolic tissues such as liver, adipose tissue, or muscle. However, PBMCs are a relevant surrogate to investigate systemic metabolic alterations, particularly in pediatric populations where invasive sampling is not feasible. The cross-sectional design limits the ability to establish causal relationships between gene expression, metabolic parameters, and added sugar intake. Additionally, dietary intake was assessed using food frequency questionnaires, which may be affected by recall bias and reporting errors, especially in children. Notably, a strength of this study is that it is among the first to examine the association between added sugar intake and the expression of genes related to metabolic disease in a pediatric population, providing insight into early molecular alterations potentially linked to diet.

5. Conclusions

Our findings indicate that the expression of the *MEG3–FTO–ATF4* axis is altered in children with obesity. Both MEG3 and FTO exhibit sex-dependent expression pattern in children without obesity, while in children with obesity, this sex-related variation is also observed for SREBP1, FASN, ACACA, FTO and MEG3, suggesting a potential role of sex in the regulation of lipid metabolism genes under obesogenic conditions. In children with IR, FAS was expression increased, whereas SREBP1, ATF4, ACACA, FTO and MEG3 expression were reduced. Moreover, added sugar intake correlated negatively with SREBP1, ACACA and FTO, suggesting a potential modulatory effect of dietary sugar on lipid metabolism gene expression. Taken together, these findings suggest that dietary sugar intake, gene expression, and sex differences may interact and contribute to early metabolic alterations in children with obesity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, Figure S1: title; Table S1: title; Video S1: title.

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Abbreviations

The following abbreviations are used in this manuscript:

ACACA	Acetyl-CoA Carboxylase ALPHA
ATF4	Activating Transcription Factor 4;
BAM	Mexican Food Composition Table
BMI	Body Mass Index
CDC	Disease Control and Prevention
CHO	Cholesterol
ENSANUT	Mexican National Health and Nutrition Survey
FASN	Fatty Acid Synthase
FFQ	food frequency questionnaire
FTO	Fat Mass and Obesity-Associated Gene
HDL-C	High-Density Lipoprotein Cholesterol
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
IR	Insulin resistance
LDL-C	Low-Density Lipoprotein Cholesterol
lncRNA	Long non-coding RNA

MEG3	Maternally Expressed Gene 3
PBMCs	peripheral blood mononuclear cells
PPARγ	Peroxisome Proliferator-Activated Receptor γ
SREBP1	Sterol Regulatory Element-Binding Protein 1
T2D	Type 2 diabetes
TG	Triglycerides

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