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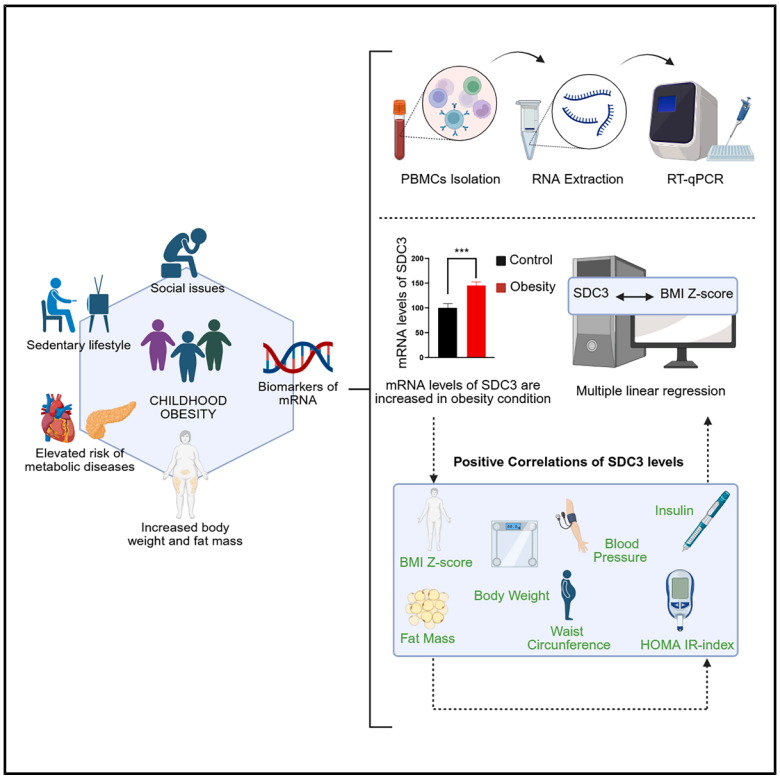
Increased levels of syndecan-3 are associated with childhood obesity

Graphical abstract



Highlights

- SDC3 gene expression might be a prognostic marker of obesity
- Pediatric population with obesity showed higher expression of *SDC3* in PBMCs
- Elevated mRNA levels of *SDC3* are associated with higher BMI *Z* score

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In brief

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Increased levels of syndecan-3 are associated with childhood obesity

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SUMMARY

Childhood obesity is considered an important risk factor for developing obesity in adult. Syndecan-3 (SDC3) has recently come into focus as a possible molecular target involved in alterations of energy homeostasis, however, its role in childhood obesity remains undefined. We measured mRNA expression levels of SDC3 in children and adolescents with obesity from peripheral blood mononuclear cells and correlating them with different anthropometric and biochemical parameters. We performed a cross-sectional study involving 143 Caucasian children and adolescents. We found increased SDC3 expression in children and adolescents with obesity. In addition, a positive statistical correlation has been found between the levels of SDC3 with body weight, BMI, BMI *Z* score, fat mass, waist circumference, insulin levels, HOMA-IR index, and maximum blood pressure. Finally, a multiple linear regression analysis indicated that the BMI *Z* score variable was the most significant. Taken together, our results highlight a still unexplored link between SDC3 and childhood obesity.

INTRODUCTION

The worldwide prevalence of obesity has increased at an alarming rate in recent decades, both in adults and children.[1,2] According to data from the WHO, from 1990 to 2022, obesity has doubled in the adult population and quadrupled among adolescents worldwide.

Childhood obesity is a disease that results from a complex interaction of several factors, including genetic, metabolic, social, and environmental components. It is estimated that genetic and/or endocrinological syndromes account for only 1% of cases of childhood obesity, with the remaining 99% corresponding to external and modifiable factors generated by a hypercaloric diet, as well as sedentary lifestyles, stress, lack of sleep, environmental factors, and socioeconomic problems, such as the difficulty to access to healthy foods at an affordable cost.[3]

Other factors that also have a significant effect on the development of childhood obesity are the genetic component derived from overweight or parents with obesity, low, or excess birth weight, as well as the absence or reduced breastfeeding.[4–6] Recent evidences also suggest that components, such as gestational age, type of birth, breastfeeding model, use of antibiotics during childbirth and early childhood can also produce an inadequate development of our digestive microbiota during the first months of life, can lead us to a state of dysbiosis with a general alteration of the microbiota, which has also been associated with the development of overweight/obesity in children.[7–9] All these factors contribute in a determinant way, during the early stages of life, in the development of obesity, so the identification of early

markers and new therapies that can help prevent or ameliorate food intake disorders in children and adolescents are of vital importance.[1] In 2023, the American Academy of Pediatrics published the clinical practise guideline for the Evaluation and Treatment of Children and Adolescents with obesity,[10] where it is stated that obesity is a chronic disease, which can be alleviated, but not cured, so the search for new markers of obesity is extremely necessary and urgent.

In this direction, peripheral blood mononuclear cells (PBMCs) are emerging as an efficient source of circulating markers for obesity.[11,12] PBMCs constitute a useful and minimally invasive source in the search for biomarkers for the study of diseases in humans.[13]

Alternatively, syndecans (SDCs) are a group of glycoproteins that belong to the family of heparan sulfate proteoglycans and are involved in the regulation of several cellular and physiological processes.[14,15] Mammals present four major isoforms of SDCs (SDC1, SDC2, SDC3, and SDC4), each exhibiting specific functions and expression patterns in different tissues and distinct metabolic functions.[16–19]

Syndecan-3 (SDC3) is a co-receptor that is predominantly expressed in the brain where it acts as a regulator of food intake and body weight by modulating the activity of the melanocortin antagonist, agouti-related peptide.[20] The endogenous physiological relevance of SDC3 was demonstrated by the fact that SDC3-deficient mice are hypophagic, lean, resistant to diet-induced obesity (DIO), and exhibit increased glucose sensitivity.[21] SDC3 has previously been linked to obesity in humans. In this regard, previous studies identified a strong association between 2 of the nonsynonymous single nucleotide polymorphism (SNPs) of SDC3 gene with obesity in the Korean population[22] and with metabolic syndrome in the Taiwanese population.[23]

Therefore, the main objective of this work is to decipher the metabolic role of the gene SDC3 in children and adolescents with obesity by measuring its messenger ribonucleic acid (mRNA) expression levels from PBMCs and correlating them with different anthropometric and biochemical parameters.

RESULTS

SDC3 gene expression is upregulated in children and adolescents with obesity

First, we show the expected increase caused by obesity in variables, such as weight, BMI, BMI *Z* score, waist circumference, total fat mass and fat mass from trunk, waist, pelvis, arm, leg, homeostasis model assessment of insulin resistance (HOMA-IR) index, insulin levels, triglycerides, maximum and minimum blood pressure, heart rate and leptin compared to control group in our population (Table 1). In contrast, other circulating factors that are considered beneficial for the disease, such as high-density lipoprotein (HDL) levels, are significantly decreased in children with obesity compared to control (Table 1).

Previous studies have linked the SDC3 gene to obesity in the adult population.[22,23] Therefore, these data support our hypothesis, suggesting that SDC3 expression levels could also be related to childhood obesity. To this end, we first determined the mRNA expression levels of SDC3 in PBMCs from samples of control and children and adolescents with obesity of both sexes. Our results show that the expression of SDC3 is significantly elevated in individuals with obesity compared to controls (Figure 1).

To determine whether there is a sexual dimorphism in SDC3 expression levels in our population, we investigated whether there are differences in SDC3 expression in both sexes in individuals with obesity. Our analysis shows that the increase in SDC3 expression associated with obesity is independent of sex (Figure 1), since there is a significant increase of SDC3 in both sexes with obesity.

To evaluate the potential implication of the stage of pubertal development on SDC3 expression, we performed an analysis based on prepubertal and pubertal stages on our childhood population (Figure 2).

Although in the prepubertal stage the increase in SDC3 levels is not significant between individuals with obesity and controls, the trend is quite clear, and similar to the pubertal group, where there is a statistically significant increase in the expression of our target. These data show a positive regulation of the SDC3 gene in the childhood population with obesity, however, it does not seem to depend on the stage of pubertal maturity of the individuals.

SDC3 gene correlates with obesity, adiposity, insulin resistance, and maximum blood pressure in children and adolescents

Once we determined that SDC3 expression is positively regulated in childhood obesity, the next step was to correlate these levels of SDC3 expression with anthropometric and biochemical factors as we indicate in the Table 2.

Our results showed a significant positive correlation between SDC3 expression levels and body weight, BMI, BMI *Z* score, waist circumference, total fat mass, trunk fat mass, waist fat mass, pelvis fat mass, arm fat mass, leg fat mass, insulin levels, HOMA-IR index, and maximum blood pressure (Figures 3A–3M).

SDC3 gene expression in obesity is explained by BMI *Z* score

To learn more about the regulation of SDC3 expression levels in our childhood population, we used a linear regression model, where we try to fit linear models between a dependent variable, SDC3 expression levels, and all the biochemical factors and anthropometric parameters as the independent variables, but only those that had a significant correlation with SDC3 expression, adjusting them, to get the best Akaike information criterion (AIC).

Our results show that after an adjusted multiple linear regression analysis of our variables, BMI *Z* score (p=<0.001) has a statistically significant contribution on the levels of circulating SDC3 expression in our childhood population (Table 3).

Interestingly, the BMI *Z* score exhibited a positive and statistically significant association with the dependent variable, SDC3 expression levels, with an average increase of 0.11 units for each unit increase in the BMI *Z* score. The 95% confidence interval (0.06–0.16) did not include zero, further supporting the presence of a real and consistent association (Table 3).

Table 1. Summary of the anthropometric and biochemical characteristics of the participants in the cross-sectional study

Parameters	Control (n = 55)	Obesity (n = 88)	p-value
Age (year)	11.2 (7.5–13.1)	12.4 (9.8–13.9)	0.1029
Weight (kg)	**42.4 (24.8–48.5)**	**65.8 (46.9–82.2)**	**<0.0001**
Height (cm)	**148.0 (125.5–157.0)**	**153.5 (137.1–161.8)**	**0.0096**
Waist to height ratio	**6.9 (6.9–8.0)**	**5.4 (3.8–7.0)**	**0.0002**
BMI (kg/m^2)	**18.1 (15.8–20.8)**	**27.0 (24.6–31.4)**	**<0.0001**
BMI *Z* score	**0.1 (-0.5–1.1)**	**3.2 (2.4–3.9)**	**<0.0001**
Waist circumference (cm)	**70.0 (57.0–80.5)**	**92.2 (82.0-102.0)**	**<0.0001**
Total fat mass (g)	**$1.7{\cdot}10^4$ ($6.5 \cdot 10^3$–$2.1{\cdot}10^4$)**	**$2.1{\cdot}10^4$ ($2.0 \cdot 10^4$–$3.1{\cdot}10^4$)**	**<0.0001**
Trunk fat mass (g)	**7098.0 (2700–$1.1{\cdot}10^4$)**	**$1.3{\cdot}10^4$ ($9.1 \cdot 10^3$–$1.8{\cdot}10^4$)**	**<0.0001**
Waist fat mass (g)	**1153.0 (421.5–1997.3)**	**2205.5 (1605.8–3085.0)**	**<0.0001**
Pelvis fat mass (g)	**2826.0 (1462.0–4151.2)**	**4410.5 (3108.2–6749.5)**	**<0.0001**
Arm fat mass (g)	**1088.0 (434.0–1920.8)**	**2145.0 (1431.2–3128.8)**	**<0.0001**
Leg fat mass (g)	**7424.0 (2801.0–8940.9)**	**9148.0 ($7.2 \cdot 10^3$–$1.5{\cdot}10^4$)**	**<0.0001**
Muscle mass (g)	**$3.0{\cdot}10^4$ ($2.2 \cdot 10^4$–$3.0{\cdot}10^4$)**	**$3.0{\cdot}10^4$ ($2.6 \cdot 10^4$–$3.6{\cdot}10^4$)**	**0.0191**
Glucose (mg/dL)	79.0 (75.0–81.0)	79.1 (73.8–84.2)	0.4445
Insulin (mUI/L)	**11.0 (5.2–13.3)**	**14.4 (9.9–20.2)**	**<0.0001**
HOMA-IR index	**2.2 (1.1–2.7)**	**2.8 (1.9–3.9)**	**<0.0001**
IGF-1 (ng/mL)	331.8 (196.5–384.0)	298.5 (232.0–426.2)	0.202
Triglycerides (mg/dL)	**52.0 (35.5–67.4)**	**65.5 (49.8–89.2)**	**0.0005**
Free fatty acids (mg/dL)	11.8 (7.5–14.3)	11.8 (10.1–13.1)	0.7499
Total cholesterol (mg/dL)	161.0 (146.5–176.0)	157.0 (135.8–175.0)	0.1856
LDL – cholesterol (mg/dL)	94.6 (84.5–102.0)	86.5 (76.8–104.0)	0.8372
HDL – cholesterol (mg/dL)	**52.0 (50.5–63.0)**	**44.0 (38.0–54.0)**	**<0.0001**
Maximum blood pressure	**110.0 (99.0–112.8)**	**114.5 (109.0–123.0)**	**<0.0001**
Minimum blood pressure	**63.0 (57.5–66.4)**	**68.0 (63.0–74.2)**	**0.0003**
Heart rate	**84.3 (74.0–88.0)**	**84.1 (75.0–91.2)**	**0.0497**
Leptin (ng)	**7.1 (1.6–10.6)**	**11.9 (7.3–15.8)**	**<0.0001**
FSH (mUI/L)	3.0 (1.4–3.4)	2.8 (0.9–4.6)	0.5308
TSH (mUI/L)	2.6 (1.7–3.1)	2.5 (2.0–3.3)	0.1363
fT3 (ng/dL)	4.3 (4.3–4.3)	4.3 (4.2–4.3)	0.8769
fT4 (ng/dL)	1.2 (1.2–1.2)	1.2 (1.1–1.2)	0.0701
Estradiol (pg/mL)	30.7 (11.8–32.1)	18.8 (11.8–32.1)	0.3266
Testosterone (ng/mL)	0.7 (0.1–0.7)	0.4 (0.1–0.7)	0.7734
Progesterone (ng/mL)	1.0 (0.8–1.0)	1.0 (0.5–1.0)	0.9685
Vitamin – D (ng/mL)	19.0 (15.0–23.0)	17.0 (13.8–22.0)	0.6469
Luteinizing hormone (UI/L)	2.1 (0.1–2.1)	1.5 (0.1–3.1)	0.2598

Values are presented as median (interquartile range). Differences between groups were assessed by Mann-Whitney test. Bold values mean significant statistical differences: weight, height, waist to height ratio, body mass index, BMI Z-score, waist circumference, total fat mass, trunk fat mass, waist fat mass, pelvis fat mass, arm fat mass, leg fat mass, muscle mass, insulin, HOMA-IR index, triglycerides, HDL – cholesterol, maximum blood pressure, minimum blood pressure, heart rate and leptin. Bold values mean significant statistical differences with a p-value <0.05.

DISCUSSION

The present work has focused on studying the relationship between SDC3 expression levels in PBMCs and obesity in pediatric populations, an area of study for which scientific evidence is still lacking.

While SDC3 levels have been shown to be increased in children with obesity, no significant differences in SDC3 expression levels were observed between sexes. Furthermore, our results also indicate that elevated SDC3 levels in obesity are independent of the prepubertal or pubertal stage of the pediatric population.

It is important to note that SDC3 was reported to be a facilitator on the actions of AgRP an orexigenic hypothalamic peptide previously.[24] SDC3 is expressed in the hypothalamus, which is known to regulate feeding behavior and body weight. Moreover, when SDC3 is inhibited in SDC3 null mice, those animals are protected against obesity and show a negative energy balance with

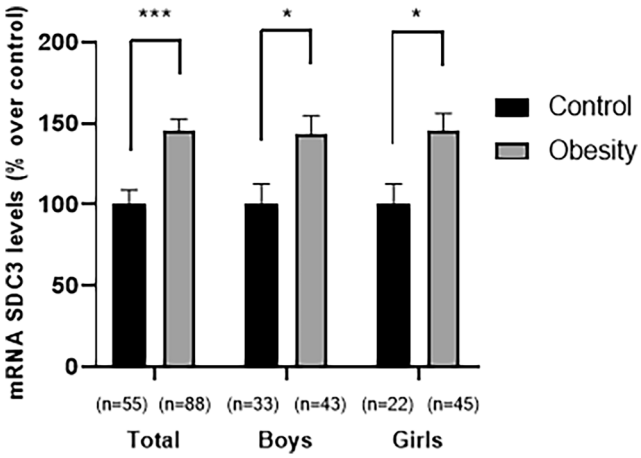

Figure 1. SDC3 mRNA levels in children and adolescents with obesity stratified by sex

Differences between groups were assessed by two-sided *Student's* t test. Data are expressed as mean ± SEM. *$p < 0.05$, ***$p < 0.001$.

reduced adipose content and improved glucose tolerance.[21] These facts support our findings and fit well with the factor that high levels of SDC3 are associated with obesity.

Using an integrated approach that combined the assessment of genetic, biochemical and anthropometric parameters, we have shown a significant correlation between SDC3 expression levels and multiple metabolic and anthropometric factors associated with obesity.

The research revealed that SDC3 mRNA expression correlates positively with most of the anthropometric variables assessed and related to obesity, such as body weight, BMI, BMI *Z* score, waist circumference, general and localized fat mass, and hormone-related variables such as insulin levels and HOMA-IR index, suggesting a potential mechanism between obesity and

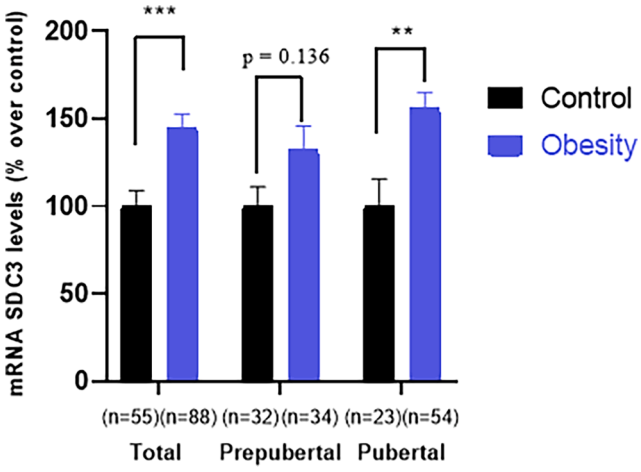

Figure 2. SDC3 mRNA levels in children and adolescents with obesity stratified by pubertal stage

Differences between groups were assessed by two-sided *Student's* t test. Data are expressed as mean ± SEM. **$p < 0.01$, ***$p < 0.001$.

increased SDC3 expression. Consequently, at the mechanistic level, a recent work has showed the role of SDC3 in the control of lipid metabolism from *in vitro* studies carried out in brain microvascular endothelial cells (BMECs).[25] Moreover, this interesting report also proposed that SDC3 could regulate fat metabolism through the AMPK/SIRT1/PPARG signal pathway, keys sensors closely related to obesity control and energy balance regulation.[26–28]

Notably, we provide scientific evidence showing the positive correlation between SDC3 with BMI, BMI *Z* score, waist circumference, and fat mass in Caucasian children population. This is consistent with our hypothesis that elevated SDC3 levels could be a biomarker of disease and implies that SDC3 correlates positively with relevant anthropometric factors associated with obesity. This is to be expected if we consider that obesity is directly proportional to the amount of fat and waist circumference and, in fact, is defined by a high BMI. Besides, we propose that SDC3 expression profiling can reveal a potential as a prognostic biomarker associated with obesity, knowing well the limitation that cross-sectional approach cannot establish cause-effect relationships and the samples of subjects were obtained at a single time point. Moreover, prognostic biomarkers are often identified from observational data, which would fit well with our study.[29–32]

Furthermore, SDC3 gene expression correlates positively with insulin, as well with HOMA-IR index. These correlations are consistent with the fact that obesity is characterized by a resistance in the action of this hormone and thus its levels are high in this pathological state. Interestingly our results are also supported by a recent study in which researchers found high levels of SDC3 in erythrocytes of diabetic mice and diabetic patients.[33] Moreover, the same study showed a positive correlation of SDC3 levels and blood glucose in the erythrocytes of diabetic mice.[33] Furthermore, this fact provides a possible endocrine target by which SDC3 could interfere with obesity. Additionally, previous studies in adults have showed a positive association of SNPs of SDC3 with obesity in Korean[22] and Taiwanese population.[23] However, although evidence of SDC3 concerning the association between SDC3 and obesity have been previously described in adults, we report that elevated levels of SDC3 are associated with obesity in Caucasian children population.

Our data also show a positive correlation between SDC3 levels and maximum blood pressure, which is conceivable if we are considering that a major comorbidity of obesity is poorer cardiovascular health and consequently the development of arterial hypertension. Interestingly, this suggests a still unexplored connection between our target SDC3 and cardiovascular pathophysiology in childhood obesity. Along this same line, a recent work in adults has identified an association of lower expression of SDC3 in peripheral blood samples from patients with hypertension but without obesity.[34] These results suggested that while closely related, probably the mechanism in obesity and cardiovascular disease can be regulated by different via mediated by SDC3.

In addition, to gain a more precise understanding of the interactions between SDC3 and various factors associated with obesity, a multiple linear regression analysis was implemented. This advanced statistical approach allowed us to adjust for several covariates simultaneously, thus isolating the specific

Table 2. Correlations were performed by Spearman's correlation coefficient

Parameters	Spearman R2	*p* value
Age (years)	0.0865	0.304
Weight (kg)	**0.2300**	**0.006**
Height (cm)	0.1196	0.155
Waist to height ratio	−0.1474	0.079
BMI (kg/m^2)	**0.3007**	**<0.001**
BMI *Z* score	**0.3453**	**<0.001**
Waist circumference (cm)	**0.2546**	**0.002**
Total fat mass (g)	**0.1870**	**0.025**
Trunk fat mass (g)	**0.2061**	**0.014**
Waist fat mass (g)	**0.2177**	**0.009**
Pelvis fat mass (g)	**0.1867**	**0.026**
Arm fat mass (g)	**0.2203**	**0.008**
Leg fat mass (g)	**0.1819**	**0.030**
Muscle mass (g)	0.0940	0.264
Glucose (mg/dL)	0.0802	0.341
Insulin (mUI/L)	**0.2317**	**0.005**
HOMA-IR index	**0.2000**	**0.017**
IGF-1 (ng/mL)	0.0409	0.628
Triglycerides (mg/dL)	0.0717	0.395
Free fatty acids (mg/dL)	0.0437	0.771
Total cholesterol (mg/dL)	−0.0301	0.721
LDL – cholesterol (mg/dL)	0.0241	0.775
HDL – cholesterol (mg/dL)	−0.1539	0.067
Maximum blood pressure	**0.1945**	**0.020**
Minimum blood pressure	0.1400	0.095
Heart rate	0.1268	0.131
Leptin (ng)	0.1200	0.154
FSH (mUI/L)	−0.0247	0.770
TSH (mUI/L)	0.1033	0.220
fT3 (ng/dL)	0.0340	0.687
fT4 (ng/dL)	−0.0740	0.380
Estradiol (pg/mL)	−0.0015	0.986
Testosterone (ng/mL)	−0.0432	0.609
Progesterone (ng/mL)	−0.0400	0.635
Vitamin – D (ng/mL)	−0.0168	0.842
Luteinizing hormone (UI/L)	−0.0185	0.826

Bold values mean significant statistical differences with a *p* value <0.05.

impact of each variable on SDC3 mRNA levels. This analysis has allowed us to study the relationship of SDC3 with all the variables selected, with BMI *Z* score being the most significant variable in the model.

In summary, this study demonstrates the relationship between increased SDC3 expression levels with different factors associated with childhood obesity, such as, body weight, BMI, BMI *Z* score, waist circumference, fat mass, insulin levels, HOMA-IR index, and maximum blood pressure in humans.

Together, our study highlights the influential role of SDC3 gene in pediatric obesity and SDC3 could be used as a promising biomarker. Although existing research on this aspect is extremely limited, we propose that our findings can reveal a potential for SDC3 as prognostic biomarker associated with obesity in children. However, further research related to mechanisms actions of SDC3 *in vivo* and pharmacotherapy related with SDC3 are not available and are needed to propose SDC3 for therapeutic interventions in the future.

Limitations of the study

One of the limitations in our study arises from impossibility of performing RNA-seq on all our samples. While RNA-seq provides a comprehensive and high-throughput analysis of gene expression, we have opted for qPCR as an alternative method, which, although more cost-effective, offers a narrower scope and lower sensitivity compared to RNA-seq. Despite these constraints, qPCR remains a valuable tool for targeted gene expression analysis, but it limits the depth of the data that we could obtain from RNA-seq.

Another limitation of our study is its cross-sectional design, which captures data at a single point in time. This approach restricts our ability to draw causal inferences or understand the temporal dynamics of gene expression changes. Without longitudinal data, we cannot assess how gene expression patterns evolve over time or in response to specific interventions. Consequently, the findings may only reflect associations rather than causal relationships.

RESOURCE AVAILABILITY

Lead contact

To request more information about the study, please contact the lead contact: Dr. Mar Quiñones (mar.quinones@usc.es).

Materials availability

This study did not create new or unique reagents.

Data and code availability

The patients' dataset used on this study is not available since it cannot be deposited in a public repository. It was strictly used for this research, and we are not allowed to make it open access. However, all the details regarding the code and packages used in R analysis have been deposited at the Github repository and are publicly available as of the date of publication. Accession numbers are listed in the key resources table. Any additional request related to the dataset can be directed to the lead contact.

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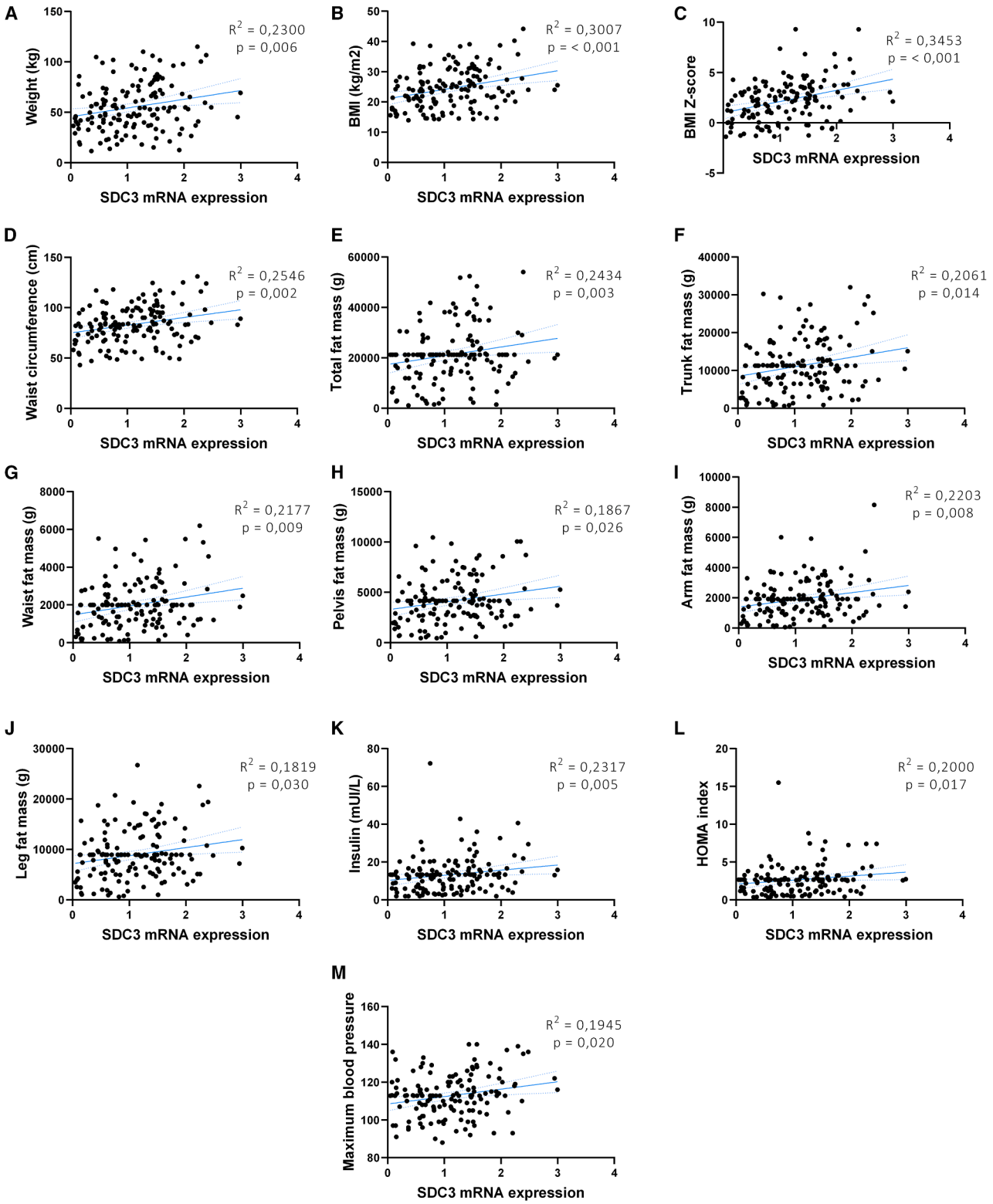

Figure 3. Correlation analysis of the study population using the Spearman's correlation coefficient

Bivariate correlation between SDC3 gene expression and body weight (A), BMI (B), BMI *Z* score (C), waist circumference (D), total fat mass (E), trunk fat mass (F), waist fat mass (G), pelvis fat mass (H), arm fat mass (I), leg fat mass (J), weight (J), insulin (K), HOMA-IR index (L), and maximum blood pressure (M).

Table 3. Adjusted multiple linear regression model

	Multiple linear regression model		Adjusted multiple linear regression model	
	Estimation (CI 95%)	*p* value	Estimation (CI 95%)	*p* value
BMI *Z* score	**0.13 (0.02–0.25)**	**0.018**	**0.11 (0.06–0.16)**	**<0.001**
AIC	283.85		265.2	

Estimation refers to the beta coefficients, the CI to the confidential interval and AIC refers to Akaike information criterion. Bold values mean significant statistical differences with a *p* value <0.05.

AUTHOR CONTRIBUTIONS

D.M.-M.: investigation, formal analysis, methodology, writing – original draft, visualization. E.P.: investigation, formal analysis, methodology, writing – review and editing, A.Y.B.: methodology, investigation, R.P.-Lois., M.B., L. D.-G.D., B.B.-G., M.V., R.P.-Leis., R.V.-C., and A.E.-G., investigation, methodology, and writing – review and editing, L.M.S., R.L., O.A.-M., and M.Q. conceptualization, resources, supervision, writing – review and editing, visualization, project administration, and funding acquisition.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
PBMCs	Investigation in Nutrition, Growth and Human Development Unit of Galicia (Clinical University Hospital of Santiago de Compostela)	Central Ethics and Research Committee of the Galician Autonomous Community (2013/256)
Critical commercial assays		
E.Z.N.A.® Cycle Pure Kit (V-spin)	Omega Bio-tek	D6492-00S
One-step NZYSpeedy RT-qPCR Green kit, ROX plus	NZYtech	MB34402
LabAssay (TM) NEFA	FUJIFILM Wako Shibayagi Corporation	633–52001
PolymorphPrep solution	Progen	1895
TRIzol reagent	Thermo Fisher Scientific	15596018
Oligonucleotides (Sequence 5'———3')		
β - actin Human Forward: CACAGAGCCTCGCCTTTGC	Thermo Fisher Scientific	N/A
β - actin Human Reverse: CCACCATCACGCCCTGG	Thermo Fisher Scientific	N/A
SDC3 Human Forward: GACATCCCTGAGAGGAGCAC	Thermo Fisher Scientific	N/A
SDC3 Human Reverse: CTTCTGGCAGCTCGAAGTCT	Thermo Fisher Scientific	N/A
Software and algorithms		
GraphPad Prism	GraphPad Software	https://www.graphpad.com/
R Studio	The R Foundation	https://posit.co/download/rstudio-desktop/
R code and packages	Github repository	https://github.com/diegomunozafk/Script_Draft_iScience/blob/main/Script_iScience.R https://doi.org/10.5281/zenodo.15781352

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

A cohort of patients was selected including a total of 143 Caucasian children and adolescents (3- 15 years old). Enrollment into the study occurred from June 2013 to the present.[12,35–37] The patients were recruited from the Unit of Investigation in Nutrition, Growth and Human Development of Galicia-Unit of Pediatric Nutrition (Clinical University Hospital of Santiago de Compostela). The participants and their families were informed about the aim of the study. This cohort was classified according to body mass index (BMI) in two groups identified as children with obesity (n = 88) or control group defined as children with normal weight (n = 55) utilizing the international BMI cut-off points.[12,38] The cohort also included the study of both sexes, for which samples of boys were used (a sample size of n=33 used as a control and n=43 with obesity) and samples of girls (a sample size of n=22 used as a control and n=45 with obesity).

The obesity group was recruited from children who came to the Unit of Investigation in Nutrition, Growth and Human Development of Galicia-Unit of Pediatric Nutrition (Clinical University Hospital of Santiago de Compostela) to confirm the diagnosis of obesity or for identification of minor gastrointestinal disorders that were discarded after clinical and laboratory tests. Therefore, the validity of the Caucasian patients included is justified because they represent the majority childhood population with obesity in the area of this study.

Inclusion criteria: Children with obesity classified according to BMI utilizing the international BMI cut-off points.[12,38] Whereas the control group were children who came to the emergency departments owing to a common infection, that were not confirmed for other pathologies.

Exclusion criteria: Children with chronic or inflammatory pathologies, a congenital disease or psychomotor disability and taking drugs for treatment of alterations in blood pressure, hormonal, glucose or lipid metabolism and those who did intensive exercise in the 24 h before the examination or had been involved in other studies 3 month before that could interfere in the results.

This is a cross-sectional study conducted in accordance with the principles outlined in the Declaration of Helsinki. All procedures were approved by the Central Ethics and Research Committee of the Galicia Autonomous Community (code 2013/256). Written informed consent was obtained from the parents of all participating children. Individuals who had taken any medication or had chronic disorders that could potentially influence the study outcomes were excluded from the study.

Because previous studies demonstrated an association between SDC3 with obesity and metabolic syndrome,[22,23] we included in this work the analysis of SDC3 with parameters closely related with these pathologies as BMI, weight, fat mass, lipid content, blood pressure, sexual hormones, thyroid hormones or D vitamin, etc. All of them have been previously related to obesity.[39–44]

METHOD DETAILS

Clinical examination and blood sampling

Anthropometric measurements were taken in the morning by a single pediatrician, with participants wearing only underwear and no shoes. Body weight was recorded using a digital electronic scale (Seca mod. 813, gmbh & CO) with 0.1 kg precision, while height was measured with a calibrated wall-mounted stadiometer (Seca mod. 213, gmbh & CO) with 0.1 cm precision. BMI was calculated by dividing the weight in kilograms by the square of the height in meters.

Waist circumference was measured in the fasting state using an inelastic tape, positioned horizontally midway between the lowest rib margin and the iliac crest while the child stood at the end of a gentle expiration. Whole-body composition was assessed to determine bone mass, non-bone fat-free mass, and fat mass using a General Electric LunarEncore® DEXA system with an X-ray source (38 keV and 70 keV photons).

Clinical examinations were conducted by trained pediatricians following standardized protocols. Pubertal stage was assessed using Tanner's criteria, where stage 1 is prepubertal and stages 2 to 5 are considered pubertal. Blood samples were collected after a 12-hour overnight fast.

Biochemical assays

Plasma glucose, total cholesterol, and triglyceride levels were assessed using the Advia 2400 Chemistry System from Siemens Healthcare Diagnostics, Erlangen, Germany. The levels of low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol were measured using the SAS-3 Cholesterol Profile kit from Helena Biosciences Europe, Gateshead, UK. Plasma leptin was determined using a commercial ELISA kit from DRG International, Springfield Township, NJ, USA.

Additionally, plasma levels of insulin, thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), oestradiol, testosterone, luteinizing hormone, follicle-stimulating hormone (FSH), and vitamin D were determined using a chemiluminescence immunoassay on the Advia Centaur XP analyzer, also from Siemens Healthcare Diagnostics, Erlangen, Germany. Plasma insulin-like growth factor 1 (IGF-1) was analysed using a chemiluminescence immunoassay on the Immulite 2000 analyzer, also from Siemens Healthcare Diagnostics, Erlangen, Germany. Lastly, circulating free fatty acids were measured using a colorimetric kit following the instructions from Wako Chemicals GmbH, Neuss, Germany.

PBMCs isolation from peripheral human blood

PBMCs were isolated from serum-free blood as previously described.[12] Briefly, the blood was diluted 1:2 with saline serum lacking calcium and magnesium. Five milliliters of this diluted blood were carefully layered onto 4 mL of PolymorphPrep solution (Progen) without disturbing the phases, followed by centrifugation at 500 x g for 30 minutes to achieve gradient separation. The PBMC-containing cell band was collected into a new tube, diluted with saline serum to a final volume of 13 mL, and centrifuged for 10 minutes at 500 x g. The cell pellet was resuspended in 13 mL of saline serum and centrifuged again for 10 minutes at 500 x g. This was followed by adding 1500 μL of saline serum and centrifuging for 10 minutes at 500 x g. Finally, the pellet was resuspended in 750 μL of TRIzol reagent.

mRNA isolation and quantification by SYBR Green One-step RT-qPCR

The mRNA was isolated from the sample resuspended in 750 μL of TRIzol reagent (15596018) by adding 150 μL of chloroform (VWR chemicals), followed by vortexing and centrifugation at 12,000 x g for 15 minutes at 4°C. The aqueous phase was carefully collected, and an equal volume of 70% ethanol (VWR chemicals) was added. The mixture was transferred to a column from the RNA extraction kit (Omega Bio Tek). After centrifugating the column at 10,000 x g for one minute at 21°C, the flow-through was discarded, and 500 μL of Wash Buffer I (RNA extraction kit, Omega Bio Tek) was added. The column was centrifuged again at 10,000 x g for one minute at 21°C, and the liquid was discarded. Then, 500 μL of Wash Buffer II (RNA extraction kit, Omega Bio Tek) was added, and the column was centrifuged at 10,000 x g for one minute at 21°C. This washing step was repeated twice. The remaining liquid was removed, and the column was centrifuged at 14,000 x g for 2 minutes at 21°C. Finally, 40 μL of RNase-free water was added, and the column was centrifuged at 14,000 x g for 2 minutes at 21°C to elute the RNA.

One-step reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed in 10 μL reaction volumes, using 10 ng of RNA template[12]. The RNA concentration was measured spectrophotometrically with a NanoDrop (Thermo Scientific NanoDrop). The reaction mixture contained 0.4 μL of 10 μM forward primer, 0.4 μL of 10 μM reverse primer, 0.4 μL of NZYRT mix, 5 μL of One-step NZYSpeedy qPCR Probe master mix (2x) (NZYtech, Lisbon, Portugal), and 1.8 μL of nuclease-free water. A calibration curve with varying concentrations was included as an amplification control.

The RT-qPCR protocol began with an initial reverse transcription step of 15 minutes at 50°C, followed by polymerase activation for 2.5 minutes at 95°C. The cycling protocol was then repeated for 40 cycles using a 96-well thermal cycler (QuantStudio 3, Applied Biosystems, Thermo Fisher Scientific) with the following conditions: 5 seconds at 95°C and 45 seconds at 60°C. This was followed by a melt curve stage consisting of 15 seconds at 95°C, 1 minute at 60°C, and 1 second at 95°C.

The primers employed were designed using the online tools provided by the PRIMER-blast program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The list of utilized primers is provided in the key resources table.

QUANTIFICATION AND STATISTICAL ANALYSIS

To describe the sample, the median and interquartile range and interquartile rank were calculated for quantitative variables. Frequencies and percentages were computed for qualitative variables. Statistical significance between groups were performed by the Mann-Whitney test. Due to the characteristics of the sample and the sample collection, the presence of missing values (NAs) was imputed by coercion (https://doi.org/10.32614/CRAN.package.dplyr).

An *a priori* power analysis was conducted for an independent samples t-test with unequal group sizes using the pwr package in R (https://CRAN.R-project.org/package=pwr). Assuming a medium effect size (Cohen's d = 0.5), a significance level of $\alpha = 0.05$, and sample sizes of 55 individuals as control and 88 as obesity, the estimated statistical power was 0.826, indicating a high probability of detecting a true effect in the study.

Correlation analysis was performed using Spearman's correlation coefficient to evaluate the relationships between groups and variables. Consequently, a multiple linear regression analysis was assessed with the variables with a significant correlation with SDC3 RNA expression. Finally, the AIC was used to assess the model that give a better adjustment. Results with a p-value minor than 0.05 were considered significant.

Moreover, all the details regarding the code and packages used in R analysis to carry out the study have been included below and in the supplemental information.

Statistical packages used to perform the statistical analysis were: GraphPad Prism software (GraphPad Software, 2023) and R software (R Core Team, 2023).

The statistical significance is indicated as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

ADDITIONAL RESOURCES

This work is not part of/involves a clinical trial.