Association of total and pancreatic serum amylase enzymatic activity with insulin resistance and the glucose and insulin responses to oral starch test in Mexican children

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Summary

Background/Objectives: Little is known about the effect of serum amylase enzymatic activity on glucose metabolism. We investigated the association of serum amylase enzymatic activity with fasting plasma glucose, insulin resistance (IR), and the plasma glucose and insulin response to an oral starch test (OST) in Mexican children.

Methods: Anthropometric data, glucose and insulin levels, and the serum enzymatic activity of total (AMYt), salivary (AMY1), and pancreatic (AMY2) amylase were analysed in 764 children ($N_{normal weight} = 427/N_{obesity} = 337$). After categorization into low (LA) and high (HA) AMYt, an OST with commercial white bread was performed in 39 children ($N_{normal weight} = 17/N_{obesity} = 22$).

Results: A positive association between serum enzymatic activity of AMY2 and IR was observed in children with obesity (p = 0.018). Children with normal weight had lower plasma glucose and insulin response to OST than children with obesity ($P_{glucose} = 4.1 \times 10^{-12}$; $P_{insulin} = 2.1 \times 10^{-15}$). Compared with the LA group, children

Daniel Locia-Morales and Miguel Vázquez-Moreno contributed equally to this study.

Eugenia Flores-Alfaro and Miguel Cruz jointly supervised this study.

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with HA showed lower plasma glucose and insulin response to OST ($P_{glucose} \le 0.040$; $P_{insulin} \le 0.015$).

Conclusion: Our results suggest that AMY2 is positively associated with IR. A high level of AMYt is related to lower glucose and insulin responses to OST in Mexican children, regardless of their weight status.

KEYWORDS

insulin resistance, Mexican children, oral starch test, serum amylase

1 | INTRODUCTION

In 2016, the World Health Organization reported that 1.9 billion adults and 39 million children had overweight and obesity worldwide. Obesity is one of the most important public health concerns due to its high prevalence and the severity of its metabolic complications. Among the main comorbidities of obesity are insulin resistance (IR), type 2 diabetes, and premature mortality.¹ Regarding pediatric population, recent studies report that Hispanic child show a higher risk of developing metabolic syndrome compared with non-Hispanic white and black children.² To date, Mexico has the largest overweight burden on the gross domestic product (GDP) in all the Organization for Economic Co-operation and Development (OECD) countries (5.6%).³ The last national survey of health and nutrition in 2020 reported the prevalence of obesity for children (5–11 years), adolescents (12 and 19 years), and adults (≥20 years) was 18.6%, 17.0%, and 40.2%, respectively.⁴

The recent increase in overweight and obesity rates in the Mexican population can be explained in great part by the consumption of hypercaloric food and sugar-sweetened beverages, in addition to reduced physical activity.^{5,6} However, biological factors such as in utero exposure, sex, age, ancestral background, pre-existing medical conditions, gut microbiome composition, epigenetics, and genetics also have a critical role in the development of obesity.⁷ As an example, the copy number (CN) variation of the salivary amylase gene (AMY1A) has been associated with childhood and adult obesity in the Mexican population.^{8,9,10} The association between CN of AMY1A and obesity also has been reported in children from Europe and North America.^{11,12}

Recently, we evidenced a positive association of CN of AMY1A/AMY2A genes with serum enzymatic activity of salivary (AMY1) and pancreatic (AMY2) amylase in Mexican children with normal weight and obesity. We also observed that the serum enzyme activity of AMY1 and AMY2 was negatively associated with obesity risk in the subset of children eating medium/high amounts of starch.¹⁰

AMY1 and AMY2 are digestive enzymes that breakdown diet starch (a polymer of numerous glucose units linked by glycosidic bonds) into oligosaccharides, maltose, and maltotriose, which are further digested by disaccharidases before being taken up as monosaccharides by enterocytes through the Na⁺-glucose cotransporter 1 (SGLT1).¹³ This is one of the main reasons why α -amylase is considered a therapeutic

target for treating and maintaining postprandial glycaemia in people with type 2 diabetes.¹⁴ Our recent findings suggest that AMY1 and AMY2 play an important role in the metabolism of carbohydrates through more efficient starch digestion, which suggests that individuals with high amylase enzymatic activity may present higher blood concentrations of glucose after starch ingestion, compared with those who have low amylase. However, Mandel et al. (2012) reported that healthy adults from United States with high amylase have lower postprandial blood glucose concentrations than individuals with low amylase.¹⁵ This observation was not confirmed in the Asian population.¹⁶ While different starchy foods are frequently consumed in the Mexican diet, this research topic has not been investigated in Mexican children so far. This prompted us to investigate in a Mexican pediatric sample: (i) the association of serum enzymatic activity of total amylase (AMYt), AMY1, and AMY2 with fasting plasma glucose (FPG) and insulin (FPI) levels, hyperinsulinemia, and IR; (ii) the plasma glucose and insulin responses to an oral starch test (OST) of children with normal weight and obesity; and (iii) whether children with low (LA) and high (HA) AMYt show differences in glucose and insulin responses to an OST (Figure 1).

2 | METHODS

2.1 | Study population

The project was approved by the ethics committee of the Instituto Mexicano del Seguro Social (CONBIOETICA-09-CEI-009-20 160 601) and was conducted in compliance with the Declaration of Helsinki. Prior to the inclusion in the study, child assent and parents/legal guardians written informed consent were obtained.

We analysed the association of serum enzymatic activity of AMYt, AMY1, and AMY2 with FPG, FPI, hyperinsulinemia, and IR in a sample of 764 volunteer children with normal glucose tolerance (NGT) (427 with normal weight and 337 with obesity) between the age of 6 and 12 from Campeche, Mexico City and Oaxaca (Table 1). The study was conducted from 2011 to October 2018.

Thirty-nine volunteer children (17 with normal weight and 22 with obesity) with NGT from Campeche were enrolled to analyse whether children with LA and HA show differences in plasma glucose and insulin responses to OST (Table 1). The study was conducted between February 2019 and December 2020. i) To analyze the association of serum enzymatic activity of AMYt, AMY1 and AMY2 with fasting plasma glucose and insulin levels, hyperinsulinemia and insulin resistance (n= 427 NW / 337 OB).



FIGURE 1 Flow chart of the study design. AMY1, serum enzymatic activity of salivary amylase; AMY2, serum enzymatic activity of pancreatic amylase; AMYt, serum enzymatic activity of total amylase; NW, normal weight; OB, obesity; LA, low serum enzymatic activity of total amylase (<60 UI/L); HA, high serum enzymatic activity of total amylase (<60 UI/L);

2.1.1 | Anthropometric and biochemical measurements

Weight and height were measured in all the participants with a digital scale (Seca, Hamburg, Germany) and a portable stadiometer (Seca 225, Hamburg, Germany), respectively. Body mass index (BMI) was calculated as weight (kg)/height (m)². BMI was converted to age- and gender-adjusted standard deviation scores (BMI-SDS) using the guide-lines from the Centers for Disease Control.¹⁷ BMI percentiles were used to classify children as being normal weight and obese, according to the Centers for Disease Control and Prevention CDC 2000 reference (18). Children with a BMI \geq 5th and < 85th percentile were classified as having normal weight, while those with a BMI \geq 95th percentile were diagnosed as having obesity. Systolic and diastolic blood pressure (SBP and DBP) were measured using a mercurial sphygmomanometer (ALPK2, Tokyo, Japan).

After at least 8–10 h of fasting, a blood sample was obtained from all the participants, and total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and FPG were measured in a COBAS Icobas 6000 modular analyser series (Hoffman-La Roche. Basel, Switzerland) by enzymatic colorimetric method. FPI was measured by chemiluminescence (IMMULITE, Siemens, USA). Homeostatic model assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-B) were calculated using the equation by Matthews et al.¹⁹ FPI values <1 μ U/mL were discarded from the study because of the risk of blood haemolysis. Since 2-h plasma glucose values were not available for the study samples, according to the 2003 American Diabetes Association criteria, children with FPG <5.6 mmol/L were classified as having NGT.²⁰ Hyperinsulinemia and IR were defined as FPI >15.05 μ U/mL and HOMA-IR >3.4, respectively.²¹

2.1.2 | Serum enzymatic amylase measurement

Serum enzymatic activity of AMYt and AMY2 were measured by enzymatic colorimetric assay, as previously described,¹⁰ using a COBAS Icobas 8000 modular analyser series (Hoffman-La Roche. Basel, Switzerland). The enzymatic activity of AMY1 was calculated by subtracting the activity of AMY2 from the activity of AMY1. Based on previous recommendations, only children with the normal range of AMYt enzymatic activity (29–99 U/L) were included in the study.²²

2.1.3 | Evaluation of plasma glucose and insulin responses to OST

To evaluate the plasma glucose and insulin responses to OST, we created groups of children with LA and HA serum enzymatic activity, based on the median of the raw data of AMYt (60 IU/L). Because OST is not a routine procedure, we followed the protocol proposed by Mandel et al (2012)¹⁵ with minor modifications to evaluate whether children with LA and HA show differences in plasma glucose and insulin responses to OST. All the children were instructed to eat commercial white wheat bread (50 g of standardized starch, Artesian white bread, Grupo Bimbo, Mexico) over the course of 15 min. Before swallowing, each participant mixed adequately the starch with saliva for a period of 5 s. To evaluate plasma glucose and insulin during OST, a certified phlebotomist placed a catheter into an antecubital vein, and blood samples were collected in tubes without additives. Between samplings, the catheter was flushed with saline to avoid clots. A blood sample was obtained 5 min before the starch ingestion (time 0), and after the starch eating, blood samples were obtained at 15, 30, 45, 60, and 120 min. All the blood samples were kept vertical for 30 min and

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TABLE 1 General characteristics of children with normal weight and obesity

Fasting plasma effect	N = 427	N = 337	p-value
Female, N (%)	250 (58.5)	153 (45.4)	0.043
Age (years)	9.0 ± 1.9	9.2 ± 1.8	0.938
BMI (kg/m ²)	16.9 ± 3.0	24.4 ± 2.7	$\textbf{7.6}\times\textbf{10}^{-\textbf{215}}$
SDS-BMI	0.24 ± 0.65	2.03 ± 0.77	$\textbf{3.0}\times\textbf{10}^{-\textbf{135}}$
SBP (mmHg) ^a	91.7 ± 15.6	99.3 ± 18.5	$\textbf{7.0}\times\textbf{10}^{-\textbf{7}}$
DBP (mmHg) ^a	68.7 ± 14.2	72.7 ± 14.4	0.002
TC (mg/dL)	150.3 ± 27.3	160.2 ± 31.5	$\textbf{5.0}\times\textbf{10^{-6}}$
HDL-C (mg/dL)	50.5 ± 10.6	42.1 ± 10.3	$\textbf{1.3}\times\textbf{10}^{-\textbf{25}}$
LDL-C (mg/dL)	91.2 ± 21.1	101.1 ± 24.9	$\textbf{5.6}\times\textbf{10}^{-\textbf{9}}$
TG (mg/dL)	80.0 ± 41.3	131.5 ± 72.2	$\textbf{4.4}\times\textbf{10}^{-\textbf{32}}$
FPG (mmol/L)	4.3 ± 0.4	4.4 ± 0.5	0.830
FPI (μU/mL) ^b	5.5 ± 3.5	13.0 ± 8.6	$\textbf{2.5}\times\textbf{10}^{-\textbf{36}}$
HOMA-IR ^b	1.1 ± 0.7	2.6 ± 1.8	$\textbf{1.9}\times\textbf{10}^{-\textbf{34}}$
HOMA-B ^b	100.0 ± 73.5	266.7 ± 491.1	$\textbf{2.5}\times\textbf{10}^{-\textbf{7}}$
AMYt (IU/L)	62.8 ± 24.0	55.5 ± 21.1	$\textbf{8.0}\times\textbf{10^{-6}}$
AMY1 (IU/L)	40.8 ± 21.8	35.3 ± 18.0	$\textbf{1.9}\times\textbf{10}^{-4}$
AMY2 (IU/L)	21.9 ± 7.0	20.1 ± 6.6	$\textbf{2.8}\times\textbf{10}^{-4}$
Hyperinsulinemia N (%) ^b	0 (0)	79 (32.6)	$\textbf{2.6}\times\textbf{10}^{-\textbf{25}}$
Insulin resistance, N (%) ^b	0 (0)	64 (26.4)	$\textbf{8.6}\times\textbf{10}^{-\textbf{26}}$
Oral starch test	N = 17	N = 22	-
Female, N (%)	9 (52.9%)	9 (40.9%)	0.458
Age (years)	8.8 ± 1.8	9.1 ± 1.7	0.150
BMI (kg/m ²)	19.5 ± 4.6	23.2 ± 3.4	0.006
SDS-BMI	0.79 ± 1.6	2.2 ± 0.85	0.001
FPG (mg/dL)	4.6 ± 0.4	4.7 ± 0.3	0.415
FPI (μU/mL)	5.8 ± 4.5	12.9 ± 7.8	0.0019
HOMA-IR	1.2 ± 0.9	2.7 ± 1.6	0.0014
HOMA-B	103.0 ± 135.7	403.4 ± 402.2	0.054
AMYt (IU/L)	63.2 ± 26.0	60.6 ± 20.0	0.740

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Note: Data are expressed as mean \pm standard deviation and *N* (%). Differences in means were analysed using Student's t tests and Chi square was employed to compare sex frequencies. Significant *p* values (*p* < 0.05) are represented in bold.

Abbreviations: AMYt, total amylase; AMY1, salivary amylase; AMY2, pancreatic amylase; BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HDL-C, high-density lipoprotein cholesterol; HOMA-B, homeostatic model assessment for β -cell function; HOMA-IR, homeostatic model assessment for insulin resistance; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; SDS, metabolic traits age- and sex-adjusted standard deviation scores; TC, total cholesterol; TG, triglycerides.

^aChildren with normal weight (n = 295) and obesity (n = 218) analysed. ^bChildren with normal weight (n = 291) and obesity (n = 242) analysed.

centrifuged at 15000 RPM for 10 min. The plasma was extracted and stored in 500 μl aliquots at $-80^\circ C.$

2.1.4 | Data analysis

Shapiro–Wilk test was used to evaluate the normal distribution of continuous variables, and rank-based normal inverse transformations were applied for the variables that did not show normality (Table S1).

Student's t and Chi² tests were used to assess the differences of continuous and categorical traits between children with normal weight and obesity. Based on the raw data, we analysed tertiles of serum enzymatic activity of AMY1 and AMY2 (low, medium, and high). Linear and logistic regression models adjusted for age, sex, and location (Campeche, Mexico City, and Oaxaca) were used to assess the association of serum enzymatic activity of AMY1, AMY1, and AMY2, as continuous and tertile variables, with continuous (FPG, FPI, HOMA-IR, and HOMA-B) and binary traits (hyperinsulinemia and IR), respectively. To evaluate differences in plasma glucose and insulin responses to OST in children with LA and HA, areas under the curve (AUC) were determined by the trapezoidal method and compared with Student's t tests. Peak of plasma glucose and insulin concentrations in the OST were also compared with Student's t tests. Statistical analyses were conducted using SPSS software (version 28.0, IBM, Armonk, NY) and two-sided p value <0.05 was considered significant. QUANTO and G*Power software were used for statistical power calculations.

3 | RESULTS

3.1 | General characteristics of the study population

The general characteristics of children included in the study are presented in Table 1. We included 427 children with normal weight and 337 with obesity regarding the association of serum enzymatic activity of AMYt, AMY1, and AMY2 with FPG, FPI, hyperinsulinemia, and IR. Age, sex ratio, and FPG did not show significant differences between normal weight and obesity groups. The values of BMI, SDS-BMI, SBP, DBP, TC LDL-C, TG, FPI, HOMA-IR, and HOMA-B were significantly higher in children with obesity. In contrast, HDL-C level, AMYt, AMY1, and AMY2 were significantly lower, compared with children with normal weight. Only children with obesity presented hyperinsulinemia (32.6%) and IR (26.4%).

In the study of plasma glucose and insulin responses to OST, we included 17 children with normal weight and 22 with obesity (Table 1). Age, sex ratio, and FPG were similar in children with normal weight and obesity. Children with obesity showed higher BMI, SDS-BMI, FPI, HOMA-IR, and HOMA-B than children with normal weight. We also compared the general characteristics between children with LA (AMYt <60 IU/L) and HA (AMYt ≥60 IU/L) separately in children with normal weight and obesity, all the anthropometric and biochemical variables were similar between HA and LA groups, except AMYt, which was significantly higher in HA than LA group.

3.2 | Association of AMYt, AMY1, and AMY2 with FPG, FPI, HOMA-IR, and HOMA-B

In a test adjusted for age, sex, and location, we did not find any significant association between serum enzymatic activity of AMYt, AMY1,

TABLE 2 Association of AMYt, AMY1, and AMY2 with hyperinsulinemia and insulin resistance in children with obesity

Continuous variables					
Traits	AMYt	AMY1	AMY2		
Hyperinsulinemia	0.998 [0.984-1.011] (0.742)	0.996 [0.981-1.011] (0.599)	1.009 [0.970-1.050] (0.659)		
Insulin resistance	1.001 [0.988-1.015] (0.845)	0.998 [0.983-1.014] (0.831)	1.024 [0.983-1.067] (0.427)		
Tertiles variables					
Traits	AMYt	AMY1	AMY2		
Hyperinsulinemia	0.974 [0.688-1.379] (0.883)	0.938 [0.664–1.327] (0.719)	1.315 [0.911-1.898] (0.144)		
Insulin resistance	4 000 [0 40/5 4 550] (0 (00)	4 00/ [0 7/0 4 547] (0 / 40)	4 504 [4 004 0 044] (0 040)		

Note: Data are expressed as OR [95% CI] (p-value). Analysis by logistic regression model adjusted for age, sex, and location. Significant p values (p < 0.05) are represented in bold.

Abbreviations: AMYt, total amylase; AMY1, salivary amylase; AMY2, pancreatic amylase.



FIGURE 2 Plasma glucose (A) and insulin (B) responses to oral starch test in children with normal weight and obesity. NW, normal weight; OB, obesity. Sample size: Normal weight = 17; Obesity = 22

	Normal weight		
Trait	LA, N = 10	HA, N = 7	<i>p</i> -value
AUC glucose	11190.0 ± 462.8	9905.0 ± 1997.0	0.04
AUC insulin	2726.0 ± 465.5	1853.0 ± 256.4	0.006
	Obesity		
Trait	LA, N = 10	HA, N = 12	p-value
AUC glucose	12720.0 ± 635.0	12075.0 ± 329.7	0.005
AUC insulin	6006.0 ± 879.0	4918.0 ± 678.7	0.015

TABLE 3 Area under the curve for plasma glucose and insulin responses to oral starch test in children with HA and LA, separately for normal weight and obesity

Note: Data are expressed as mean \pm standard deviation. Differences in means were analysed using Student's t tests. Significant *p* values (*p* < 0.05) are represented in bold.

Abbreviations: AUC, area under curve; LA, low total amylase; HA, high total amylase.



FIGURE 3 Plasma glucose (A),(C) and insulin (B),(D) responses to oral starch test in children with LA and HA, separately by normal weight and obesity status. LA, low total amylase; HA, high total amylase. Sample size: Normal weight LA = 10; Normal weight HA = 7; Obesity LA = 10; Obesity HA = 12

and AMY2, as a continuous variable, and FPG, FPI, HOMA-IR, and HOMA-B in children with normal weight ($P \ge 0.139$) and obesity ($P \ge 0.429$) (Table S3). We then created tertiles of low (<47.3 IU/L), medium (47.3–65.2 IU/L), and high (>65.2 IU/L) AMYt, low (<27.7 IU/L), medium (27.7–44.4 IU/L), and high (>44.4 IU/L) AMY1,

and low (<17.8 IU/L), medium (17.8–22.9 IU/L), and high (>22.9 IU/L) AMY2. We tested their association with FPG, FPI, HOMA-IR, and HOMA-B adjusted for age, sex, and location in children with normal weight and obesity analysed separately (Table S3). We did not observe any significant association (all $P \ge 0.114$, Table S3).

3.3 | Association of AMYt, AMY1, and AMY2 with hyperinsulinemia and IR

We only observed hyperinsulinemia and IR in children with obesity. As a result, we excluded children with normal weight from the association study of serum amylase enzymatic activity of AMYt, AMY1, and AMY2, as continuous and tertile variables, with hyperinsulinemia and IR (Table 2). Only tertiles of serum enzymatic activity of AMY2 were positively associated with IR (OR = 1.594; 95% confidence interval [CI] 1.084–2.344; p = 0.018; logistic regression test adjusted for age, sex, and location). We did not find any other association (all $P \ge 0.144$; Table 2).

3.4 | Plasma glucose and insulin responses to OST in children with normal weight and obesity

To evaluate the plasma glucose and insulin responses to OST, we compared the total AUC for plasma glucose and insulin values between children with normal weight and obesity. Children with normal weight showed significantly lower mean total AUC for plasma glucose and insulin levels compared with their obesity counterparts (glucose: normal weight = 11134.0 ± 439.7 vs. obesity = 12636.0 ± 480.4 , $p = 4.1 \times 10^{-12}$; insulin: normal weight = 2092.0 ± 450.3 vs. obesity = 5596.0 ± 1034 , $p = 2.1 \times 10^{-15}$, Figure 2). All plasma insulin concentration peaks and peaks of plasma glucose concentration at 30, 45, 60, and 120 min were significantly lower in children with normal weight than with obesity (Figure 2 and Table S4).

3.5 | Plasma glucose and insulin responses to OST in HA and LA groups

We also evaluated the plasma glucose and insulin response to OST in children with HA and LA, separately analysing children with normal weight and obesity (Table 3 and Figure 3). We found lower plasma glucose and insulin responses to OST in HA than LA group, in children with normal weight ($P_{Glucose} = 0.040$; $P_{Insulin} = 0.006$; Table 3 and Figure 3) and with obesity ($P_{Glucose} = 0.005$; $P_{Insulin} = 0.015$; Table 3 and Figure 3). In Table S5, we evidenced differences in plasma glucose and insulin concentration peaks between HA and LA during the OST in children with normal weight and obesity.

4 | DISCUSSION

Our findings in the Mexican pediatric population suggest (i) a positive association between tertiles of serum enzymatic activity of AMY2 and IR in children with obesity; (ii) a lower plasma glucose and insulin response to OST in children with normal weight than in children with obesity; and (iii) that high levels of AMYt are associated with lower glucose and insulin responses to OST than low levels of AMYt in children with normal weight and with obesity.

Previous studies have shown negative associations between serum amylase and markers of glucose metabolism.^{11,23,24} Nakaiima et al. reported a negative association between AMYt and fasting glucose levels in adults from Japan.²³ Also, in Japanese adults, Muneyuki et al. evidenced a negative association between AMYt and FPI, HOMA-IR, and HOMA-B.²⁴ In addition, Bonnefond et al. reported negative associations between AMY1, FPG, and HOMA-B, and between AMY2, FPG, FPI, and HOMA-B in the French adult population.¹¹ Our results show a positive association between tertiles of AMY2 and IR in Mexican children with obesity for the first time. Although the specific molecular mechanisms by which insulin promotes the increase AMY2 mRNA and protein levels are still unclear, a possible explanation for the positive association between tertiles of AMY2 and IR may be related to the fact that the IR status is compensated by increased insulin secretion and the insulinotropic action.²⁴ In animal models, the increase of insulin secretion stimulates the exocrine pancreatic function and induces both mRNA and protein levels to increase of AMY2.^{25,26}

The negative association between serum amylase and markers of glucose metabolism has been controversial in the literature. Ikeda et al. reported a positive association between salivary AMY1, FPG, and HOMA-IR in Japanese women.²⁷ AMY1 measured in saliva samples has been proposed as a reliable marker of autonomic nervous system activity in stress.²⁸ Sex differences in emotional and physiological responses to social stress have been reported in addition to sex differences in the pathophysiology of IR.^{29,30,31}

Due to its association with chronic low-grade inflammation, lipotoxicity, hyperinsulinemia, and IR, obesity is the main risk factor for impaired glucose tolerance and type 2 diabetes.³² BMI and body fat mass are negatively associated with β-cell function, insulin sensitivity. and glucose uptake assessed by oral glucose tolerance test (OGTT) in NGT children, regardless of ethnicity.^{33,34,35,36,37} Although little is known about the glucose response to OST in children, we evidence in a Mexican pediatric population that NGT children with normal weight show significant lower glucose and insulin responses to OST than NGT children with obesity. In addition, we evidenced that regardless of obesity status, children with high levels of AMYt show lower plasma glucose and insulin responses to OST than children with low levels of AMYt. Our results are similar to what Mandel et al. (2012) reported regarding healthy adults from United States adults with high amylase serum enzymatic activity in saliva who presented significantly lower blood glucose concentrations than individuals with low amylase after starch ingestion.¹⁵

Although the biological root by which amylase improves glucose metabolism is unclear, three possible mechanisms may explain it.

The first mechanism relies on the fact that pancreatic amylase binds specifically to glycoprotein N-glycans on the brush border membrane, resulting in inhibition of glucose uptake by SGLT1 and decreased intestinal glucose absorption, which presumably allows a better control of the postprandial blood glucose level.^{38,39}

The second mechanism is related to better intestinal absorption and metabolism of glucose. Recently, studies in humans and animal models have evidenced that maltose, produced by the amylolytic 8 of 10 WILEY-Pediatric

activity of AMY1 and AMY2, stimulates sweet taste enterocyte receptors (T1R2/T1R3) and facilitates a better intestinal glucose absorption through the up-regulation of enterocyte expression of SGLT1 and glucose transporter GLUT2.^{40,41,42} Additionally, amylase and/or its derived peptides may enhance the intestinal metabolism of glucose in porcine models.⁴³ The supplementation with amylase or with its derived peptides 1 h before and together with the glucose solution administrated to duodenal-jejunal bypassed pigs showed lowering effects on glucose levels in samples collected from the jugular and portal veins, which could indicate an enhanced intestinal metabolism of glucose and lower intestinal absorption to the visceral portal blood.43

The third mechanism proposes that amylase could play an important role in regulating glucose utilization through a non-insulindependent pathway with an additional decretin effect. The administration of pancreatic amylase in a healthy grow porcine model, and in exocrine pancreas of insulin-insufficient pigs resulted in lower insulin response and improved glucose disposal to an intravenous glucose test.^{43,44} Furthermore, the glucose-stimulated insulin release and the chronic insulin secretion were significantly suppressed in a rat pancreatic beta-cell line (BRINBD11) exposed to an acute and chronic (24 h) treatment of amylase, respectively.⁴⁵

Our finding of an association between high levels of serum enzymatic activity of AMYt and lower glucose AUC in response to OST may be related to better glucose absorption, promoted by maltose through more efficient starch digestion, which could be the first important step to improve the intestinal glucose metabolism, as shown in the porcine model. Moreover, the possible glucose consumption through an insulin-independent pathway could support the lower insulin AUC response to the OST observed in children with normal weight and with obesity from our study. Additionally, the possible "decretin" effect reported in the BRINBD11 cell line suggests that serum enzymatic amylase level may play an important role as a biomarker and a relevant target for the prevention and treatment of obesity, insulin resistance, and type 2 diabetes, especially in populations consuming high relative amounts of dietary starch.

Regarding lifestyle intervention, various efforts have been made to avoid an unfavourable metabolic condition. Although these are good, they have not been entirely efficient. As an example, a 2 years high protein/low glycaemic index dietary intervention showed no effect on the IR rate in European adolescents with overweight and obesity.⁴⁶ On the contrary, a recent study in pediatric Latino population evidence that a 12-week nutritional and physical activity intervention improves the OGTT-glucose phenotypes in adolescents with obesity.⁴⁷ In this way, further lifestyle trial studies could be considering the serum enzymatic amylase level to investigate whether amylase mediates the association of the lifestyle patterns with IR, insulin sensitivity, and/or β-cell function. Another important consideration for future amylase studies concerning glucose metabolism markers is the pancreatic fat accumulation, which was recently evidenced that is associated with increased FPG in children with non-alcoholic fatty liver disease.⁴⁸ Furthermore, the evaluation of the relationship between amylase and the gut microbiome composition, which have

demonstrated to be highly associated with obesity and fasting triglycerides and insulin levels in Mexican children,⁴⁹ could contribute with important insights about the possible clinical implications in the obesity and metabolic treatment.

The present study presents several strengths. This is the first report of a positive association between tertiles of AMY2 and IR in Mexican children with obesity. Using an OST, we confirm that children with normal weight present lower plasma glucose and insulin responses and demonstrate that regardless of body weight status, high levels of AMYt are associated with lower plasma glucose and insulin responses to OST. Our study also presents some limitations. We acknowledge the modest sample size of children with IR. The statistical power was sufficient to detect an association between tertiles of serum enzymatic activity of AMY2 and insulin resistance (total sample of children with obesity) and a significant effect of serum enzymatic activity of AMYt (classified as low and high enzymatic activity groups) on plasma glucose level (in children with normal weight) and insulin (children with normal weight and obesity) level responses to the OST (Tables S6, S7 and S8). In addition, we only analysed AMYt in the OST. Furthermore, we did not evaluate the effect of AMYt on plasma glucose and insulin response to OGTT, which prevents us to determinate sensitivity insulin index related to pancreatic beta-cell function. We also acknowledge that correction for multiple tests was not applied (significant p values of <0.05 were considered significant) in this study. Replication of our significant findings in an independent cohort of Mexican children is therefore granted.

In conclusion, our data evidence for the first time a positive association between tertiles of serum enzymatic activity of AMY2 and IR in Mexican children with obesity and an association between high levels of serum enzymatic activity of AMYt and lower glucose and insulin responses to OST in Mexican children with normal weight and with obesity.

AUTHOR CONTRIBUTIONS

Daniel Locia-Morales, Miguel Vázquez-Moreno, and Miguel Cruz designed the study; Roxana González-Dzib, Aleyda Pérez-Herrera, Roberto J. Robles-Ramírez, Alberto Rocha-Cruz, David Meyre, Eugenia Flores-Alfaro, and Miguel Cruz conducted the research; Daniel Locia-Morales, Miguel Vázquez-Moreno, Eugenia Flores-Alfaro, and Miguel Cruz analysed the data; Daniel Locia-Morales, Miguel Vázquez-Moreno, David Meyre, and Miguel Cruz designed tables and figures and wrote the manuscript; Roxana González-Dzib, Aleyda Pérez-Herrera, Roberto J. Robles-Ramírez, Alberto Rocha-Cruz, David Meyre, and Eugenia Flores-Alfaro critically reviewed the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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