Causal Association of Haptoglobin With Obesity in Mexican Children: A Mendelian Randomization Study

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Context: Little is known about the association between haptoglobin level and cardiometabolic traits. A previous genome-wide association study identified rs2000999 in the *HP* gene as the stronger genetic contributor to serum haptoglobin level in European populations.

Objective and Design: We investigated the association of *HP* rs2000999 with serum haptoglobin and childhood and adult obesity in up to 540/697 and 592/691 Mexican cases and controls, respectively. Anthropometric and biochemical data were collected. Serum haptoglobin was measured by an immunoturbidimetry assay. *HP* rs2000999 was genotyped using the TaqMan technology. Mendelian randomization analysis was performed using the Wald and inverse variance weighting methods.

Results: Haptoglobin level was positively associated with childhood and adult obesity. *HP* rs2000999 G allele was positively associated with haptoglobin level in children and adults. *HP* rs2000999 G allele was positively associated with childhood but not adult obesity. The association between *HP* rs2000999 and childhood obesity was removed after adjusting for haptoglobin level. In a Mendelian randomization analysis, haptoglobin level genetically predicted by *HP* rs2000999 showed a significant causal effect on childhood obesity by the Wald and inverse variance weighting methods.

Conclusion: Our data provide evidence for the first time for a causal positive association between serum haptoglobin level and childhood obesity in the Mexican population. Our study contributes

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Abbreviations: BMI, body mass index; CNV, copy number variant;DBP, diastolic blood pressure; FPG, fasting plasma glucose;FPI, fasting plasma insulin;GWAS, genome-wide association study;HDL-C, high density lipoprotein cholesterol; HOMA-B, homeostatic model assessment for beta-cell function; HOMA-IR, homeostatic model assessment for insulin resistance; LDL-C, low density lipoprotein cholesterol; HP, haptoglobin gene;SDS-BMI, BMI standard deviation scores adjusted for age and gender;SBP, systolic blood pressure; SNP, single nucleotide polymorphism;TC, total cholesterol;TG, triglyceride.

to the genetic elucidation of childhood obesity and proposes haptoglobin as an important biomarker and treatment target for obesity. (*J Clin Endocrinol Metab* 105: e2501–e2510, 2020)

Freeform/Key Words: serum haptoglobin, obesity, serum lipids, *HP* rs2000999 polymorphism; Mendelian randomization, Mexican children and adults

Obesity prevalence has nearly tripled since 1975. While the rise in obesity is a global phenomenon, certain countries are more affected than others (1). As an illustration, the Organization for Economic Co-operation and Development reported that Mexico had the second highest rate of obesity in the world in 2018, after the United States. The 2016 national survey of health and nutrition of Mexico reported obesity prevalence of 15.3%, 13.9%, and 33.3%, respectively, among children from 5 to 11 years, 12 to 19 years, and adults (2). Obesity is an important risk factor for multiple comorbidities (e.g., type 2 diabetes, cardiovascular disease, cancer) and premature all-cause mortality, and the risk increases even more for early-onset cases (3-5). While therapeutic options are available for children and adults (e.g., lifestyle and behavioral modifications, pharmaceuticals, bariatric surgery), they have limited impact on weight loss, or are only accessible for small fraction of eligible patients with obesity (6, 7). Therefore, while efforts have been made to tackle childhood and adult obesity in Mexico, they have so far failed at curbing the obesity epidemic (2, 8-10). More research is needed on the causes and consequences of obesity in this high-risk population, in order to improve prediction, prevention, and care (11, 12).

Obesity results from the complex interplay of environmental, societal, behavioral and biological influences (13). While the influence of *in utero* environment, age, and sex on obesity risk is well-documented (13), the role of other biological factors is less understood. For instance, literature provides conflicting and insufficient data on whether inflammation is a cause or consequence of obesity (14-16).

Human haptoglobin is a glycoprotein essentially synthesized by the liver (17). Haptoglobin is up-regulated by cytokines and abundantly produced during acute phase inflammation (17). Haptoglobin binds to circulating free hemoglobin released by hemolysis or normal red blood cell turnover (18). The circulating haptoglobin/hemoglobin complexes are then eliminated by Kupffer's cell(s) in the liver, which prevents the generation of reactive oxygen species and the loss of iron (18). Haptoglobin also binds apolipoprotein A-I and E, and consequently modifies the high-density lipoprotein cholesterol (HDL-C) function and cholesterol esterification (19, 20). Mouse studies have shown that haptoglobin is expressed in adipocytes, is regulated by peroxisome proliferator-activated receptor (PPAR) gamma, and regulates changes in adipocyte size and adipogenesis (21-23). Haptoglobin is encoded by the *HP* gene that is located on chromosome 16q22.2. The *HP* gene exists as 2 copy number variants (CNVs), Hp1 and Hp2 (24). The Hp1/Hp2 CNV has been associated with serum haptoglobin and lipid levels and inflammatory markers (24- 27). More recently, a genome-wide association study (GWAS) identified the single nucleotide polymorphism (SNP) rs2000999 in *HP* as the stronger genetic predictor of serum haptoglobin level, explaining 11.8% of its variation in European populations (27). Rs2000999 was also associated with total and low-density lipoprotein cholesterol and was only marginally correlated with the Hp1/Hp2 CNV in the same study (27). However, several questions remain. Are the associations of *HP* rs2000999 with serum haptoglobin and lipid levels transferable to non-European populations? Is *HP* rs2000999 associated with inflammation-related conditions such as obesity? What is the causal chain of events linking circulating haptoglobin to cardiometabolic traits? This gap in knowledge prompted us to investigate the association of *HP* rs2000999 with serum haptoglobin, obesity, and cardiometabolic traits in the Mexican population.

Subjects and Methods

Participants

In the discovery case control study, 447 children (256 with normal-weight and 191 with obesity) between the ages of 6 and 12 were enrolled in 3 different States of Mexico (Campeche, Oaxaca, and Mexico City). The study was conducted from 2017 to October 2018. As part of the National Obesity Network Mexico initiative, we also collected genetic and anthropometric data in a replication case control study of 790 children (441 with normal weight and 349 with obesity) between the ages of 6 and 12 enrolled in 12 States of Mexico (Baja California Sur, Estado de Mexico, Guanajuato, Hidalgo, Michoacán, Nayarit, Nuevo León, Puebla, Queretaro, Sinaloa, Sonora and Tamaulipas; Replication sample). The recruitment was conducted from June 2016 to October 2018.

A total of 1283 unrelated Mexican adults were enrolled in this study. Of these participants, 592 were normal weight and 691 were obese. Cases and controls

were recruited from primary health care facilities in Mexico City and from the central blood bank of the National Medical Center "Siglo XXI" in Mexico City.

Written informed consent was obtained from each adult subject prior to participation in the study. Child assent was obtained and parents (or legal guardians) provided written informed consent prior to enrollment into the study. Research was approved by local institutional review boards and was conducted in accordance with the relevant guidelines and regulations of the Declaration of Helsinki (28).

Anthropometric and biochemical measurements

All participants were weighed using a digital scale (Seca, Hamburg, Germany). Height was measured with a portable stadiometer (Seca 225, Hamburg, Germany). Body mass index (BMI) was calculated as weight (kg) / height $(m)^2$. BMI was converted to age- and gender-adjusted standard deviation scores (SDS-BMI) in children using the guidelines from the Centers for Disease Control (CDC) (29). Age- and gender- specific BMI percentiles were calculated according to the CDC 2000 reference to classify children as normal weight (BMI between the 5th to 85th percentile) and obese (BMI at or above the 95th percentile), respectively (30). In adults, normal weight and obesity were defined as a BMI < 25 kg/m^2 and BMI ≥ 30 kg/m^2, respectively. Systolic and diastolic blood pressure (SBP and DBP) were measured using a mercurial sphygmomanometer (ALPK2, Tokyo, Japan). Blood pressure readings were taken for each participant twice on the right arm in a sitting position with 5 minutes of rest between each measurement and the mean of the 2 readings was calculated. Blood samples were obtained following a 12-hour fast and were analyzed for fasting plasma glucose (FPG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) using the ILab 350 Clinical Chemistry System (Instrumentation Laboratory IL Barcelona Spain). Fasting plasma insulin (FPI) was measured by chemiluminescence (IMMULITE, Siemens, USA) and homeostatic model assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-B) were calculated using the equation by Matthews et al (31). Due to the risk of blood hemolysis, fasting insulin values < 1 µU/mL were discarded from the study. Glycemic status (normal glucose tolerance, impaired fasting glucose, impaired glucose tolerance, diabetes) was diagnosed using the American Diabetes Association criteria (32). In the absence of an oral glucose tolerance test (OGTT) 2-hour plasma glucose value, normal glucose tolerance was defined as FPG ≤ 5.6 mmol/L. Serum haptoglobin was measured by immunoturbidimetry assay using a COBAS Icobas 8000 modular analyzer series (Hoffman-La Roche. Basel, Switzerland) and Roche reagents following the manufacturer's instructions, in the CENAREM core laboratory facility (Mexico City).

DNA extraction and genotyping

Genomic DNA of all participants was isolated from peripheral blood using the AutoGenFlex STAR (AutoGen, Holliston, MA, USA), and purity and integrity were verified by 260/280 nm measurements (BioTek Instruments, Winooski, VT) and by electrophoresis in 0.8% agarose gels stained with ethidium bromide. Genotyping of rs2000999 of *HP* was performed by real-time polymerase chain reaction (RT-PCR) using TaqMan allelic discrimination assay C__11439054_10 (Thermo Fisher Scientific) on a 7900HT Fast Real-Time PCR system (Applied Biosystems, CA), following standard protocols. Genotype discrimination was evaluated using the SDS software (Applied Biosystems, CA). In children, genotyping call rates of 99.2% and 98.4% were observed for *HP* rs2000999 in the discovery and replication samples, respectively. No deviation from Hardy-Weinberg equilibrium was observed for *HP* rs2000999 in case and control groups of the discovery and replication samples (*P* value between 0.246 and 0.926; Supplementary Table 1) (33). Genotypes were duplicated in 10% of the discovery and replication samples and a genotyping concordance rate of 100% was observed. We also compared the allele frequencies of the discovery and replication samples with the adult Mexican-American reference population from the 1000 Genomes Project (1000G; Supplementary Table 1) (33). Allele frequencies in the discovery and replication samples were not significantly different from the reported frequencies in the 1000G. In adults, a genotyping call rate of 98.7% was observed for *HP* rs2000999. No deviation from Hardy-Weinberg equilibrium was observed for *HP* rs2000999 in case and control groups (*P* value of 0.70 and 0.78, respectively). Genotypes were duplicated in 10% of the sample and a genotyping concordance rate of 100% was observed. Allele frequencies in the adult sample were not significantly different (*P* = 0.07) from the reported frequencies in the Mexican-American reference population from 1000G.

Data analysis

The normal distribution of continuous variables was tested using the Shapiro-Wilk test. For the traits that significantly deviate from normality, rank based inverse normal transformations were applied (Supplementary

Table 2) (33). Differences between cases and controls for continuous and categorical traits were tested with Student *t* and Chi-squared tests, respectively. Linear and logistic regression models adjusted for age, sex, and glycemic status in some analyses were used to assess associations. Additional adjustments for obesity status and/or serum haptoglobin level were performed for associations with cardiometabolic traits. The genetic analyses with *HP* rs2000999 were performed under the additive model considering the minor allele as the effect allele, as previously described (27). The ratio of coefficients method, also called Wald method, was used to perform Mendelian randomization tests for binary outcomes and the result was confirmed by inverse variance weighting method (34). We did not perform association studies between serum haptoglobin level, *HP* rs2000999, and BMI / SDS-BMI as a continuous trait. Obesity case control study designs are not compatible with these analyses and hence they may result in biased estimations of the effects (35). We followed the strategy reported previously by Ronald J. Feise and considered independent Bonferroni corrections for each question asked (36). QUANTO software was used for statistical power calculations. The statistical analyses were conducted using SPSS software (version 22.0, IBM, Armonk, NY).

Results

Characteristics of the child study population

Anthropometric and cardiometabolic traits of the participants from the discovery and replication samples are presented in Table 1. We enrolled 256 children with normal weight and 191 children with obesity in the discovery sample. Age and sex ratio were not significantly different in the normal-weight and obesity groups. Children with obesity had on average 7.6 kg/m^2 higher BMI and 1.7 higher SDS-BMI unit than their normal-weight counterparts. All the other metabolic traits were significantly

Table 1. General Characteristics of Mexican Children With Normal Weight and Obesity

Trait	Normal weight	Obesity	*P* Value
Discovery sample	N = 256	N = 191	-
Female, N (%)	130 (50.8)	84 (44)	0.154
Age (years)	9.1 ± 1.9	9.0 ± 1.7	0.539
BMI (kg/m^2)	16.7 ± 1.8	24.3 ± 3.0	**3.9×10^{-35}**
SDS-BMI	0.318 ± 0.749	1.992 ± 0.108	**5.4×10^{-28}**
WHR	0.845 ± 0.060	0.924 ± 0.065	**2.9×10^{-22}**
SDS-WHR	0.238 ± 0.899	0.902 ± 0.957	**8.1×10^{-17}**
SBP (mmHg)[a]	98.0 ± 8.6	106.2 ± 12.4	**4.4×0^{-10}**
SDS-SBP[a]	0.170 ± 0.768	0.623 ± 1.108	**1.6×10^{-11}**
DBP (mmHg)[a]	63.2 ± 8.1	67.5 ± 10.4	**1.7×10^{-4}**
SDS-DBP[a]	0.241 ± 0.887	0.293 ± 1.133	**1.8×10^{-5}**
LDL-C (mg/dL)	91.1 ± 22.1	98.2 ± 23.4	**0.001**
HDL-C (mg/dL)	50.2 ± 10.1	43.1 ± 10.7	**7.7×10^{-12}**
TC (mg/dL)	151.1 ± 27.3	159.2 ± 30.9	**0.004**
TG (mg/dL)	82.5 ± 41.9	128.2 ± 61.6	**7.7×10^{-19}**
FPG (mmol/l)	4.7 ± 0.4	4.7 ± 0.4	0.460
FPI (μU/ml)[b]	5.3 ± 3.1	11.5 ± 7.9	**2.4×10^{-21}**
HOMA-IR[b]	1.0 ± 0.6	2.2 ± 1.6	**5.1×10^{-18}**
HOMA-B[b]	95.3 ± 64.9	247.5 ± 233.1	**4.4×10^{-23}**
Haptoglobin (mg/dL)	77.8 ± 45.2	105.7 ± 47.5	**6.8×10^{-10}**
HP rs2000999 G/G, N (%)	175 (68.4)	145 (75.9)	0.107
HP rs2000999 G/A, N (%)	70 (27.3)	43 (22.5)	
HP rs2000999 A/A, N (%)	11 (4.3)	3 (1.6)	
Replication sample	N = 441	N = 349	-
Female, N (%)	215 (48.8)	206 (59.0)	**0.004**
Age (years)	9.0 ± 2.0	9.1 ± 1.7	0.228
BMI (kg/m^2)	16.7 ± 1.7	25.2 ± 3.6	**2.5×10^{-31}**
SDS-BMI	0.249 ± 0.770	1.993 ± 0.059	**6.1×10^{-23}**
HP rs2000999 G/G, N (%)	283 (64.2)	247 (70.8)	0.124
HP rs2000999 G/A, N (%)	144 (32.7)	95 (27.2)	
HP rs2000999 A/A, N (%)	14 (3.2)	7 (2.0)	

Data are expressed as mean ± standard deviation and N (%). Difference in sex ratios was analyzed using chi-squared test. Differences in means were analyzed using Student *t* tests. Significant *P* values ($P < 0.05$) are represented in bold.

Abbreviations: 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; WHR, waist to hip ratio.

[a]Children with normal weight (n = 173) and obesity (n = 104) analyzed. [b]Children with normal weight (n = 150) and obesity (n = 95) analyzed.

elevated in children with obesity compared to their normal-weight counterparts, except for HDL-C, which was significantly reduced. We enrolled 441 children with normal weight and 349 children with obesity in the replication sample. Age was similar in the children with normal weight and those with obesity, but 10.2% more girls were observed in the obese group. Children with obesity had on average 8.5 kg/m^2 higher BMI and 1.4 higher SDS-BMI unit than their normal-weight counterparts.

Association of serum haptoglobin with childhood obesity and cardiometabolic traits

Sex and age were not associated with serum haptoglobin in children with normal weight (P_{sex} = 0.992; P_{age} = 0.709) and obesity (P_{sex} = 0.219; P_{age} = 0.358). Therefore, we did not stratify subsequent analyses for sex and age. Serum haptoglobin level was 77.8 ± 45.2 mg/dL in children with normal weight, and 105.7 ± 47.5 mg/dL in children with obesity ($P = 6.8 \times 10^{-10}$, Table 1). Serum haptoglobin level was positively associated with childhood obesity status in the discovery sample (OR = 1.014; 95% confidence interval [CI] 1.009-1.018]; $P = 6.55 \times 10^{-9}$; test adjusted for age, sex and recruitment center) (Fig. 1). We also tested the association between serum haptoglobin level and additional cardiometabolic traits, adjusted for age, sex, obesity status, and recruitment center. Serum haptoglobin level was nominally ($P < 0.05$) associated with HDL-C, TC, FPI, and HOMA-IR (Table 2), but most of the associations did not remain significant after Bonferroni correction for multiple tests, with the notable exception of HDL-C and FPI. We then investigated if obesity status modulates the association between serum haptoglobin level and cardiometabolic traits (Table 2). We found significant interactions between obesity status and serum haptoglobin level on LDL-C (β = –3.376 ± 1.109; P = 0.001), TC (β = –4.592 ± 1.408; P = 0.001) and TG (β = -9.318 ± 2.600; P = 0.001) (Table 2). In subsequent subgroup analyses, we only observed a significant negative association between serum haptoglobin level and LDL-C and TC in children with obesity (β = –0.117 ± 0.035; P = 0.001 and β = –0.183 ± 0.046; $P = 9.7 \times 10^{-5}$, respectively), while the effect on TG was significant both in children with normal weight and those with obesity, with an opposite direction of effect ($\beta_{normal\ weight}$ = 0.199 ± 0.075; P = 0.009 and $\beta_{obesity}$ = –0.195 ± 0.081, P = 0.017; tests adjusted for age, sex and recruitment center) (Table 3).

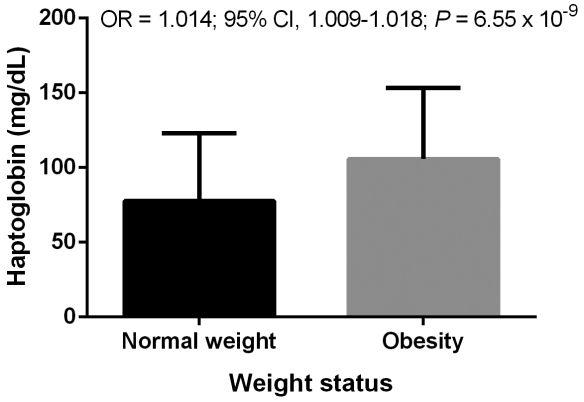

Figure 1. Association of serum haptoglobin with childhood obesity in 447 Mexican children (discovery sample). Logistic regression was adjusted for age, sex, and recruitment center.

Association of rs2000999 of HP with haptoglobin level, childhood obesity, and cardiometabolic traits

HP rs2000999 G allele was positively associated with serum haptoglobin level in the discovery sample (β = 28.904 ± 3.867; $P = 5.2 \times 10^{-13}$) adjusted for age, sex, obesity status, and recruitment center (Fig. 2). No interaction was found between *HP* rs2000999 and obesity status on serum haptoglobin level (P = 0.369).

HP rs2000999 G allele showed a positive association with obesity in both discovery and replication sample (OR = 1.487; 95% CI, 1.024-2.159; P = 0.037 and OR = 1.325; 95% CI, 1.009-1.739; P = 0.043, respectively; tests adjusted for age, sex, and recruitment center). This association was confirmed when we analyzed the discovery and replication samples together (OR = 1.390; 95% CI, 1.113-1.736; P = 0.0037; tests adjusted for age, sex, and recruitment center).

HP rs2000999 G allele was nominally ($P < 0.05$) associated with LDL-C and TC in the discovery sample (tests adjusted for age, sex, obesity status, and recruitment center) (Table 4), but the associations did not remain significant after Bonferroni correction for multiple tests. We then investigated potential interactions between obesity status and *HP* rs2000999 G allele on cardiometabolic traits in the discovery sample (Table 4). Significant interactions were observed for LDL-C (β = –2.989 ± 1.127; P = 0.008) and TC (β = –3.426 ± 1.441; P = 0.018) (Table 4). We then evaluated the effect of *HP* rs2000999 G allele on LDL-C and TC adjusted for age, sex, and recruitment center in children with normal weight and obesity separately. A negative association between *HP* rs2000999 G allele, LDL-C and TC was only observed in children with obesity (β = –12.982 ± 3.479; $P = 2.5 \times 10^{-4}$ and β = –15.578 ± 4.616; P = 0.001, respectively) (Table 3).

Causal effect of serum haptoglobin level on childhood obesity

To investigate potential causality in the associations between serum haptoglobin level and childhood

Table 2. Association of Serum Haptoglobin Level With Cardiometabolic Traits in 447 Mexican Children

		Main Effect		Interaction	
Traits	**N**	**Haptoglobin level**	***P* value**	**Haptoglobin level × obesity**	***P* value**
WHR	447	0.000 ± 0.001	0.745	0.004 ± 0.004	0.311
SDS-WHR	447	0.000 ± 0.001	0.784	0.073 ± 0.057	0.202
SBP (mmHg)	277	0.002 ± 0.012	0.976	-0.007 ± 0.604	0.991
SDS-SBP	277	0.000 ± 0.001	0.849	-0.028 ± 0.056	0.615
DBP (mmHg)	277	0.006 ± 0.011	0.593	-0.145 ± 0.543	0.789
SDS-DBP	277	0.001 ± 0.001	0.452	-0.021 ± 0.062	0.742
LDL-C (mg/dL)	447	-0.026 ± 0.024	0.279	-3.376 ± 1.109	**0.001**
HDL-C (mg/dL)	447	-0.031 ± 0.011	**0.004**	0.317 ± 0.511	0.536
TC (mg/dL)	447	-0.068 ± 0.030	0.023	-4.592 ± 1.408	**0.001**
TG (mg/dL)	447	0.021 ± 0.056	0.701	-9.318 ± 2.600	**0.001**
FPG (mmol/l)	447	0.001 ± 0.000	0.082	-0.022 ± 0.022	0.321
FPI (μU/ml)	245	0.020 ± 0.007	**0.004**	-0.313 ± 0.338	0.355
HOMA-IR	245	0.004 ± 0.001	0.007	-0.061 ± 0.071	0.389
HOMA-B	245	0.623 ± 0.348	0.075	25.231 ± 17.084	0.141

Data are expressed as β ± SE. Linear regression model was adjusted for age, sex, obesity status and recruitment center. Significant *P* values (main effect: $P < 0.0045$ after Bonferroni correction [0.05/11], and interaction: $P < 0.05$) are represented in bold.

Abbreviations: 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; WHR, waist to hip ratio.

obesity, LDL-C and TC we used a Mendelian randomization approach in the discovery sample. We first evaluated the pertinence to use *HP* rs2000999 as instrumental variable in the model. For that, we carried out an association analysis between *HP* rs2000999 and childhood obesity, LDL-C, and TC with and without adjustment for serum haptoglobin level in the model, in addition to age, sex, and recruitment center (Supplementary Table 3) (33). The association between *HP* rs2000999 and childhood obesity was removed when we added serum haptoglobin level as a covariate in the regression model ($P = 0.966$). In contrast, adjustments for LDL-C or TC did not change the association between *HP* rs2000999 and childhood obesity ($P = 0.020$ and 0.024, respectively). Furthermore, the association of *HP* rs2000999 with LDL-C and TC did not sensibly change after adjusting for serum haptoglobin level, both in the whole sample ($P_{LDL\text{-}C} = 0.016$ and $p_{TC} = 0.053$) and in the obese group ($p_{LDL\text{-}C} = 0.006$ and $p_{TC} = 0.027$) (Supplementary Table 3) (33). Based on these results, we investigated further the causal effect of serum haptoglobin level on childhood obesity risk. We performed a Mendelian randomization analysis with *HP* rs2000999 and serum haptoglobin level as instrumental and exposure variable. Serum haptoglobin level genetically predicted by *HP* rs2000999 showed a significant causal effect on obesity using the Wald method (OR = 1.621; 95% CI, 1.156-2.274; $P = 0.005$). We confirmed this significant effect by inverse variance weighting method (OR = 1.069; 95% CI, 1.005-1.137; $P = 0.040$) (Fig. 3).

Replications in the Mexican adult population

Anthropometric and cardiometabolic traits of the adult participants are presented in Supplementary Table 4 (33). In the haptoglobin level replication sample (202 adults with normal weight and 193 with obesity, all participants with normal glucose tolerance), HDL-C was significantly lower in adults with obesity, while BMI, TG, and FPG were significantly elevated. Age and sex ratio were not significantly different in the normal-weight and obesity groups. In the *HP* rs2000999 replication sample (592 adults with normal weight and 691 with obesity), all anthropometric and cardiometabolic traits were significantly elevated in the obesity group, except for HDL, which was significantly reduced. Adult participants with obesity had 13.2% more women and were on average 3.9 years older than participants with normal weight.

In the haptoglobin level replication sample ($N_{normal\ weight} = 202$, $N_{obesity} = 193$), serum haptoglobin level was not associated with sex and age in adults with normal weight ($P_{sex} = 0.22$; $P_{age} = 0.95$) and obesity ($P_{sex} = 0.14$; $P_{age} = 0.71$). Therefore, we did not stratify subsequent analyses for sex and age. Serum haptoglobin level was 108.7 ± 38.9 mg/dL in adults with normal weight, and 132.8 ± 43.1 mg/dL in adults with obesity ($P = 1.1 \times 10^{-8}$; Supplementary Table 4) (33). Serum haptoglobin level was positively associated with adult obesity (OR = 1.015; 95% CI, 1.010-1.021; $P = 7.2 \times 10^{-8}$; test adjusted for age, sex and recruitment center). We did not find any significant association between serum haptoglobin level and additional cardiometabolic traits, adjusted for age, sex, obesity status, and recruitment center (Supplementary Table 5) (33).

Table 3. Association of Serum Haptoglobin Level and *HP* rs2000999 G Allele With LDL-C, TC and TG in Children With Normal Weight and Obesity

	Main Effect of Serum Haptoglobin			
Traits	**Normal weight N = 256**	***P* value**	**Obesity N = 191**	***P* value**
LDL-C (mg/dL)	0.044 ± 0.031	0.159	−0.117 ± 0.035	**0.001**
TC (mg/dL)	0.020 ± 0.039	0.616	−0.183 ± 0.046	**9.7×10^{-5}**
TG (mg/dL)	0.199 ± 0.075	**0.009**	−0.195 ± 0.081	**0.017**
	Main Effect of *HP* rs2000999 G Allele			
Traits	**Normal weight N = 256**	***P* value**	**Obesity N = 191**	***P* value**
LDL-C (mg/dL)	−1.530 ± 2.531	0.546	−12.982 ± 3.479	**2.5×10^{-4}**
TC (mg/dL)	−2.142 ± 3.137	0.495	−15.578 ± 4.616	**0.001**

Data are represented as β ± SE. Analysis by linear regression model adjusted for age, sex and recruitment center. Significant *P* values ($P < 0.05$) are represented in bold.
Abbreviations: LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

HP rs2000999 G allele was positively associated with serum haptoglobin level in 202 normal-weight and 193 obese adults (β = 24.559 ± 4.142; $P = 6.7 \times 10^{-9}$; test adjusted for age, sex, obesity status, and recruitment center). No interaction was found between *HP* rs2000999 and obesity status on serum haptoglobin level ($P = 0.248$). The association between *HP* rs2000999 G allele and adult obesity was not significant in the haptoglobin level ($N_{normal\ weight} = 202$, $N_{obesity} = 193$; OR = 1.164; 95% CI, 0.765-1.770; $P = 0.48$; test adjusted for age, sex, and recruitment center) and *HP* rs2000999 replication samples ($N_{normal\ weight} = 592$, $N_{obesity} = 691$; OR = 0.988; 95% CI, 0.784-1.246; $P = 0.92$; tests adjusted for age, sex, recruitment center, and glycemic status). We also tested the association between *HP* rs2000999 and additional cardiometabolic traits in the *HP* rs2000999 replication sample ($N_{normal\ weight} = 592$, $N_{obesity} = 691$; tests adjusted for age, sex, obesity status, recruitment center, and glycemic status; Supplementary Table 6) (33). *HP* rs2000999 G allele was nominally associated with lower LDL-C (β = −3.892 ± 4.616; $P = 0.05$), and significantly associated with lower HDL-C (β = −1.926 ± 0.688; $P = 0.005$) and TC (β = −8.047 ± 2.452; $P = 0.001$). No significant interaction between *HP* rs2000999 and obesity status on cardiometabolic traits was observed ($P \geq 0.283$).

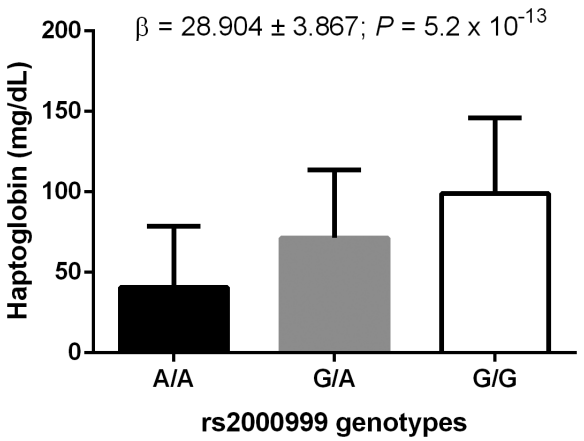

Figure 2. Association of *HP* rs2000999 with haptoglobin level in 447 Mexican children (discovery sample). Logistic regression was adjusted for age, sex, obesity status, and recruitment center.

Discussion

In 2012, a GWAS in European populations identified *HP* rs2000999 as the main genetic contributor to serum haptoglobin level (27). Our study evidenced a strong association between *HP* rs2000999 and serum haptoglobin level in Mexican children and adults and confirmed the transferability of the European GWAS signal in this population. In line with our results, a similar association between rs2000999 and serum haptoglobin level was recently reported in Chinese and Japanese adults (37, 38). SNP rs2000999 is located in the intronic region of *HP* gene and it modulates expression levels of the Hp mRNA in human subcutaneous adipose tissue (27). These data suggest that this SNP, or a SNP in very strong linkage disequilibrium with this one, is indeed functional. The transferability of the association of *HP* rs2000999 with serum haptoglobin levels in 3 ethnic groups also favors the view that this SNP may have a causal role.

Our observational and genetic data demonstrate for the first time a causal positive association between serum haptoglobin level and childhood obesity in the Mexican population. The contribution of haptoglobin to adipocyte differentiation and morphology in mouse models is consistent with these results (21-23). In addition, Chiellini et al previously reported that serum haptoglobin level was positively correlated with BMI in 312 Italian adults of European ancestry (39). In contrast, no study has

Table 4. Association of *HP* rs2000999 G Allele With Cardiometabolic Traits in 447 Mexican Children

		Main Effect		Interaction	
Traits	**N**	***HP* rs2000999**	***P* value**	***HP* rs2000999 × Obesity**	***P* value**
WHR	447	0.001 ± 0.007	0.808	0.003 ± 0.004	0.483
SDS-WHR	447	0.020 ± 0.105	0.916	0.024 ± 0.059	0.682
SBP (mmHg)	277	−0.184 ± 1.099	0.867	0.218 ± 0.599	0.716
SDS-SBP	277	−0.030 ± 0.103	0.771	0.014 ± 0.056	0.809
DBP (mmHg)	277	−0.087 ± 0.992	0.930	0.467 ± 0.544	0.391
SDS-DBP	277	−0.016 ± 0.113	0.890	0.057 ± 0.062	0.363
LDL-C (mg/dL)	447	−5.460 ± 2.057	0.008	−2.989 ± 1.127	**0.008**
HDL-C (mg/dL)	447	−0.794 ± 0.952	0.403	−0.311 ± 0.525	0.555
TC (mg/dL)	447	−6.739 ± 2.626	0.011	−3.426 ± 1.441	**0.018**
TG (mg/dL)	447	1.708 ± 4.870	0.726	1.115 ± 2.688	0.679
FPG (mmol/l)	447	0.069 ± 0.041	0.096	0.016 ± 0.023	0.469
FPI (μU/ml)	245	−0.254 ± 0.635	0.690	0.273 ± 0.342	0.426
HOMA-IR	245	−0.021 ± 0.133	0.875	0.061 ± 0.072	0.399
HOMA-B	245	−54.597 ± 31.679	0.086	9.480 ± 17.038	0.578

Data are expressed as β ± SE. Linear regression model adjusted for age, sex, obesity status and recruitment center. Significant *P*-values (main effect: $P < 0.0045$ after Bonferroni correction [0.05/11], and interaction: $P < 0.05$) are represented in bold.

Abbreviations: 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; WHR, waist to hip ratio.

investigated the association of *HP* rs2000999 with obesity traits in literature. While a positive association between serum haptoglobin level and obesity was demonstrated both in Mexican children and adults, we were unable to replicate the association between *HP* rs2000999 and childhood obesity in our adult sample. Possible explanations for this lack of replication include winner's curse effects, suboptimal statistical power, gene-by-age interactions, and birth cohort effects (13, 40, 41). However, the significant association of the *HP* rs2000999 G allele with BMI in 794 018 white-Caucasian adults from the GIANT consortium and UK Biobank ($\beta = -0.0115 \pm 0.0021$; $P = 2.5 \times 10^{-8}$) and 158 284 East Asian adults from the Biobank Japan ($\beta = 0.0129 \pm 0.0037$; $P = 4.7 \times 10^{-4}$) does not exclude a causal association between serum haptoglobin level and adult obesity.

A negative association at the observational and genetic level between serum haptoglobin level and LDL-C and TC was evidenced in Mexican children, but this association is restricted to the obesity group and is unlikely to be causal. As an illustration, the association between *HP* rs2000999 and TC and LDL-C observed in Mexican obese children is not modified when serum haptoglobin level is added as a covariate to the regression model. Haptoglobin regulates the function (rather than the expression) of apolipoprotein A-I and E, HDL-C, and cholesterol (19, 20). It may therefore be expected not to find an association between *HP* rs2000999 / serum haptoglobin level and TC and LDL-C levels in Mexican normal-weight children. In contrast, childhood obesity results in chronic low-grade inflammation and dyslipidemia (14, 42). Obesity has been shown to upregulate haptoglobin expression in adipocytes through inflammation (21, 43). The high level of haptoglobin in presence of obesity, especially in *HP* rs2000999 G allele carriers, may protect from the negative cardiometabolic consequences of reactive oxygen species, impact adipocyte metabolism and lipid function, and indirectly result in TC and LDL-C changes in Mexican obese children (18-20, 23, 44). While we were unable to find an association between serum haptoglobin and lipid levels in Mexican adults, *HP* rs2000999 G allele was associated with lower serum HDL-C, TC, and LDL-C values independent of obesity status in this population. These findings highlight the probable absence of causality between serum haptoglobin and lipid level variations in Mexican adults. The impact of the *HP* rs2000999 G allele on the generation of reactive oxygen species in mitochondria, adipocyte development and morphology, apolipoprotein A-I and E binding, cholesterol esterification, and the modulation of inflammatory

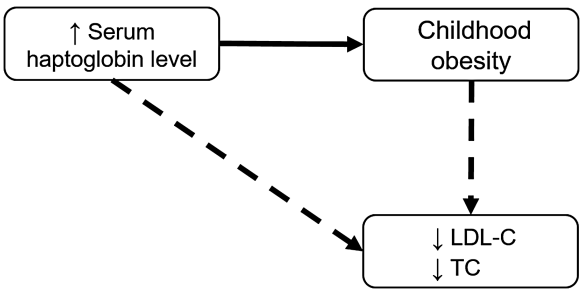

Figure 3. Causal and non-causal associations between serum haptoglobin level, childhood obesity, LDL-C (low-density lipoprotein cholesterol), and TC (total cholesterol). Plain and dotted arrows represent causal and non-causal associations, respectively.

markers represent exciting perspectives of research to precise the mechanisms linking haptoglobin to obesity and lipids in children and adults (17-23).

This report presents several strengths. This is the first study to evidence an association between *HP* rs2000999 and serum haptoglobin in the Mexican population. Using Mendelian randomization, we also demonstrate for the first time that haptoglobin is causally involved in the development of childhood obesity. While the association between *HP* rs2000999 and lipid levels has been previously reported in diverse populations (27, 45, 46), our data extend this observation to Mexican children and adult populations and suggest that this association may be modulated by obesity status. However, we acknowledge that the present study is not without limitations. The statistical power to detect an association between serum haptoglobin level and obesity was adequate in our child and adult samples (Supplementary Table 7) (33). In contrast, the statistical power to detect an association between *HP* rs2000999 and childhood and adult obesity was more modest(Supplementary Table 8) (33). Therefore, the association between serum haptoglobin level and *HP* rs2000999 with obesity in the Mexican population needs to be confirmed and extended to children and adults from diverse ethnic groups. Finally, the biological mechanisms underlying these associations have yet to be elucidated.

In conclusion, our data provide evidence for a causal positive association between serum haptoglobin level and childhood obesity in the Mexican population, as well as a negative association at the observational and genetic level between serum haptoglobin level and LDL-C and TC restricted to Mexican obese children (Fig. 3). Our study contributes to the genetic elucidation of childhood obesity and proposes haptoglobin as an important biomarker and treatment target for obesity. Our findings of this association need to be confirmed in the Mexican population and generalized to diverse populations of children and adults.

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Additional Information

Data Availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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