Leptin partially mediates the association between early-life nutritional supplementation and long-term glycemic status among women in a Guatemalan longitudinal cohort

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ABSTRACT

Background: Early-life exposure to improved nutrition is associated with decreased risk of diabetes but increased risk of obesity. Leptin positively correlates with adiposity and has glucose-lowering effects, thus it may mediate the association of early-life nutrition and long-term glycemic status.

Objectives: We aimed to investigate the role of leptin in the differential association between early-life nutrition and the risks of obesity and diabetes.

Methods: We analyzed data from a Guatemalan cohort who were randomly assigned at the village level to receive nutritional supplements as children. We conducted mediation analysis to examine the role of leptin in the associations of early-life nutrition and adult cardiometabolic outcomes.

Results: Among 1112 study participants aged (mean ± SD) 44.1 ± 4.2 y, 60.6% were women. Cardiometabolic conditions were common: 40.2% of women and 19.4% of men were obese, and 53.1% of women and 41.0% of men were hyperglycemic or diabetic. Median (IQR) leptin concentration was 15.2 ng/mL (10.2–17.3 ng/mL) in women and 2.7 ng/mL (1.3–5.3 ng/mL) in men. Leptin was positively correlated with BMI (Spearman's ρ was 0.6 in women, 0.7 in men). Women exposed to improved nutrition in early life had 2.8-ng/mL (95% CI: 0.3, 5.3 ng/mL) higher leptin and tended to have lower fasting glucose (–0.8 mmol/L; –1.8, 0.2 mmol/L, nonsignificant) than unexposed women. There were no significant differences in leptin (–0.7 ng/mL; –2.1, 0.8 ng/mL) or fasting glucose (0.2 mmol/L; –0.5, 0.9 mmol/L) in men exposed to improved nutrition in early life compared with unexposed men. Leptin mediated 34.9% of the pathway between early-life nutrition and fasting glucose in women. The mediation in women was driven by improved pancreatic β-cell function. We did not observe the mediation effect in men.

Conclusions: Leptin mediated the glucose-lowering effect of early-life nutrition in women but not in men. *Am J Clin Nutr* 2020;00:1–10.

Keywords: life cycle, first 1000 days, nutritional status, leptin, glucose, insulin resistance, mediation analysis, homeostasis model assessment

Introduction

The period from conception to 2 y of age (the first 1000 d) is a critical window of early-life development (1). Nutritional status during this window has been reported to affect health status in later years. Suboptimal nutrition in the first 1000 d is associated with increased risk of obesity, type 2 diabetes, and cardiovascular diseases (1–4). This cluster of interwoven cardiometabolic diseases is an emerging contributor to the global disease burden and is becoming increasingly prevalent in low- and middle-income countries (5). For instance, recent data from Guatemala ranked cardiovascular diseases and diabetes as the first and third leading causes of mortality, respectively, and together they account for one-third of total deaths (5).

Early-life nutritional exposure affects long-term cardiometabolic health through epigenetic, pathophysiological, and other mechanisms (1). Many of these mechanisms are not well understood. From a developmental perspective, early-life nutrition affects the ontogeny of metabolically active tissues (6). Animal models have provided relevant evidence: malnutrition caused structural and functional changes in the placenta and metabolic organs, and these changes were associated with long-term cardiometabolic disturbances (7). In human studies, it is challenging to distinguish the impact of early-life nutrition

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Supplemental Figures 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Data described in the article, code book, and analytic code will be made available upon request, pending approval by the principal investigator of the study.

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Abbreviations used: HOMA-B, HOMA of pancreatic β-cell function; INCAP, Institute of Nutrition of Central America and Panama; SES, socioeconomic status; T2DM, type 2 diabetes mellitus.

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from other determinants of cardiometabolic perturbations. Longitudinal cohort studies are valuable resources in meeting this challenge (8). More than 40 y after a nutrition trial, Ford et al. (9) reported that although early-life exposure to improved nutrition was associated with reduced risk of diabetes, odds of obesity were increased. Previous research explored the linkages between early-life nutrition and long-term risks of increased adiposity (10). However, the same factors could not explain the observed reduction in diabetes risk (9).

Cardiometabolic diseases including obesity and diabetes share underlying biochemical pathways (11). Leptin, an adipose-tissue derived hormone, is proportional to body fat mass. Leptin also participates in the programming of obesity through a leptin-dependent feedback loop (12). Leptin concentration in early life is important in the development of metabolic profile (13). Leptin is a key signaling molecule in glucose homeostasis (14–16). As a catabolic agent in metabolism, leptin reduces hepatic gluconeogenesis by limiting delivery of substrates to the liver (14, 15). It has an impact on skeletal muscle and other peripheral tissues to increase glucose uptake (15). Leptin can also regulate glucose homeostasis through pancreatic-secreted hormones (17). Because of the versatile functions of leptin, it is important to investigate the specific mechanisms it is involved in—whether leptin mainly reduces insulin resistance (assessed by HOMA-IR) or improves pancreatic β-cell function (HOMA-B) (18).

We therefore assessed the contribution of leptin to glucose homeostasis in a group of adults who participated in a randomized nutritional supplementation trial in early life. We postulated that leptin might help explain the differential effects of early-life nutrition on long-term risks of diabetes and obesity.

Methods

Study population

From 1 January, 1969 to 28 February, 1977, investigators at the Institute of Nutrition of Central America and Panama (INCAP) carried out a randomized controlled trial in 4 villages in southeastern Guatemala. Details of the initial trial and successive follow-up studies have been reported elsewhere (9, 19). Briefly, participants in 4 villages were randomly assigned to receive either *atole* (the treatment group) or *fresco* (the control group) twice daily for the duration of the study. *Atole* is a protein- and energy-containing supplementation, whereas *fresco* is a low-energy drink with no protein. A total of 2392 children were included because they either were aged <7 y at study launch or were born during the original study period. In the follow-up conducted from 2015 to 2017, 1661 cohort members (69.4% of the original cohort) were eligible for participation. The remaining cohort members had died (15.4%), emigrated (10.4%), or were lost to follow-up (4.7%). Of the 1661 eligible cohort members, 500 (30.1%) could not be contacted or declined to participate in this wave. An additional 49 (2.9%) individuals were excluded during the current wave because they either did not attend scheduled clinical exams, did not have the plasma samples required for this set of analyses, or were pregnant or lactating at the time of data collection. The final sample size was 1112 (**Supplemental Figure 1**). As previously reported, the loss-to-follow-up at this examination was not differential in terms of the random assignment (9).

Data collection

Cohort members were invited to attend centralized clinics (1 in each study site) after an overnight fast. After obtaining informed consent in Spanish, trained phlebotomists collected venous blood in EDTA-coated tubes from each participant. Blood samples were kept on ice and centrifuged at $1008\times g$ for 10 min at 4°C within 2 h of collection. On the day of sample collection, we divided plasma samples into aliquots and stored them at –20°C. Once a month, these samples were transported on dry ice to INCAP headquarters in Guatemala City, where we assayed fasting and postprandial glucose concentrations (mg/dL, converted to mmol/L for analysis) using enzymatic colorimetric methods (Cobas C111 analyzer, Roche). The remaining plasma samples were immediately stored at –80°C, shipped on dry ice in 3 installments to Atlanta, GA, and stored at –80°C until analysis. For laboratory assays conducted in the Biomarker Core Laboratory (Foundation for Atlanta Veterans Education and Research, Atlanta Veterans Affairs Medical Center), samples were thawed at 4°C over a weekend in batches of 40 participants. The plasma samples were randomly assigned into 28 batches, balanced by location of data collection, village of birth at the beginning of the INCAP Longitudinal Study, and timing of exposure to the nutritional supplements to prevent overlaying potential systematic bias in the study design with bias in the laboratory batches. We assayed insulin (mIU/L) using immunoturbidimetric methods (Kamiya Biomedical Company). We assayed fasting leptin (ng/mL) in duplicates by ELISA (Boster Biological Technology). For quality assurance, we repeated the assays for samples with implausible values, usually between 1 and 8 samples per batch (2.5–20%), and the frequency was once every other week. Overall, across all batches, ∼5% of all samples were reanalyzed. We plotted the concentrations to identify outliers within each batch and examined batch effects. In addition, we performed quality checks collectively for the first half and the second half of the data by examining their comparability, and by identifying outliers and rerunning the selected samples.

Anthropometry.

Trained research staff measured the body weight (kg), height (cm), and waist circumference (cm) of all study participants in duplicates using standardized methods. BMI was calculated as kg/m^2. Waist-to-height ratio was calculated as waist (cm) divided by height (cm). Body composition was assessed using the deuterium oxide (D_2O) dilution technique (20) (Fourier transform-infrared spectroscopy, Shimadzu 8400S). Total body water was determined based on mathematical models from the D_2O dilution, and fat-free mass was calculated using a hydration constant of 0.732 (21). Fat mass, calculated as the difference between body mass and fat-free mass, is presented as body fat percentage.

Cardiometabolic outcomes.

We focused on obesity, central obesity, hyperglycemia, and type 2 diabetes mellitus (T2DM) to characterize cardiometabolic status in the study population. Obesity was defined as BMI ≥ 30. Central obesity was defined as waist circumference ≥88 cm for women and ≥102 cm for men (22). Hyperglycemia was defined

TABLE 1 Selected characteristics of the study population by sex[1]

Characteristics	Women ($n = 674$)	Men ($n = 438$)
Sociodemographic characteristics		
Age, y	44.2 ± 4.3	43.9 ± 4.1
Exposure to *atole* in the first 1000 d	22.4	21.9
Maternal height, cm	148.9 ± 5.0	149.0 ± 4.9
Maternal age at childbirth, y	26.8 ± 7.0	27.1 ± 7.4
Maternal education level, y	1.2 ± 1.6	1.4 ± 1.7
Socioeconomic status tertiles in childhood		
Poorest	34.3	31.7
Middle	33.7	33.3
Wealthiest	32.1	34.9
Socioeconomic status tertiles in 2015		
Poorest	32.5	33.3
Middle	34.7	30.4
Wealthiest	32.8	36.3
Total grades completed, y	3.3 ± 2.2	3.6 ± 2.1
Residing in Guatemala City	18.3	19.2
Anthropometry		
Height, cm	151.5 ± 5.3	163.9 ± 6.1***
BMI, kg/m^2	29.3 ± 5.3	26.6 ± 4.2***
Obese	40.2	19.4***
Waist circumference, cm	101.8 ± 12.4	94.2 ± 10.2***
Central obesity	89.7	21.0***
Waist-to-height ratio	0.7 ± 0.1	0.6 ± 0.1***
Body fat, %	42.2 ± 5.9	28.8 ± 6.7***
Glycemic conditions		
Hyperglycemia	36.6	31.8
Type 2 diabetes	16.5	9.2***
Diabetes medication	9.5	4.3**
Biomarkers		
Fasting insulin, mIU/L	14.7 [9.1–22.2]	9.6 [6.2–16.4]***
Fasting glucose, mmol/L	5.6 [5.2–6.1]	5.4 [5.2–5.8]**
HOMA-IR	3.9 [2.3–6.3]	2.4 [1.5–4.4]***
HOMA-B	135.0 [82.6–199.4]	93.1 [61.2–152.0]***
Fasting leptin, ng/mL	15.2 [10.2–17.3]	2.7 [1.3–5.3]***

[1] Values are means ± SDs, percentages, or medians [IQRs] for continuous variables with skewed distributions. Total sample size is 1112, except for the following variables: maternal height ($n = 883$, missing 20.6%), maternal age at childbirth ($n = 1094$, missing 1.6%), and maternal education ($n = 1073$, missing 3.5%). Obesity is defined as BMI ≥ 30 kg/m^2. Hyperglycemia is defined according to the American Diabetes Association diagnostic criteria as a fasting plasma glucose concentration of 100–125 mg/dL or 2-h postchallenge plasma glucose concentration of 140–199 mg/dL among participants not reporting use of diabetes medication. HOMA-IR = fasting insulin (μIU/L) × fasting glucose (mmol/L)/22.5. HOMA-B = 20 × fasting insulin (μIU/L)/fasting glucose (mmol/L) − 3.5. *P* values were based on Student's *t* test between men and women, and independent 2-group Mann–Whitney *U* tests were used to compare biomarkers between men and women. **,***Significant difference: $^{**}P < 0.01$, $^{***}P < 0.001$. HOMA, the homeostasis model assessment; HOMA-B, HOMA of pancreatic β-cell function; HOMA-IR, HOMA for insulin resistance.

as fasting plasma glucose concentration ≥100 mg/dL and ≤125 mg/dL, or 2-h postchallenge plasma glucose concentration ≥140 mg/dL and <200 mg/dL among participants who were not using diabetic medication (23). (The 2-h postprandial plasma glucose concentration was obtained after a mix-component meal challenge designed to mimic an oral-glucose-tolerance test.) T2DM was defined as a fasting plasma glucose concentration ≥126 mg/dL, postchallenge glucose concentration ≥200 mg/dL, or use of diabetes medication (23). We calculated HOMA-IR as the product of fasting glucose (mmol/L) and fasting insulin (mIU/L) divided by 22.5, and HOMA-B as the product of fasting insulin and 20, divided by the value of fasting glucose minus 3.5 (24).

Statistical analysis

We had >80% statistical power to detect a medium effect size (Cohen's $d = 0.5$) for the difference-in-difference exposure variable for all biological markers. We described the sociodemographic characteristics of the population, pooled and separately by sex. We used Student's *t* test or the Mann–Whitney *U* test, when appropriate, for comparisons of characteristics (sociodemographic information, cardiometabolic risk factors, and biomarker concentrations) between male and female participants. We treated missingness for the following variables using bootstrapped multiple imputation: maternal height (missing 20.6%), maternal age at childbirth (missing 1.6%), and maternal schooling (missing 3.5%).

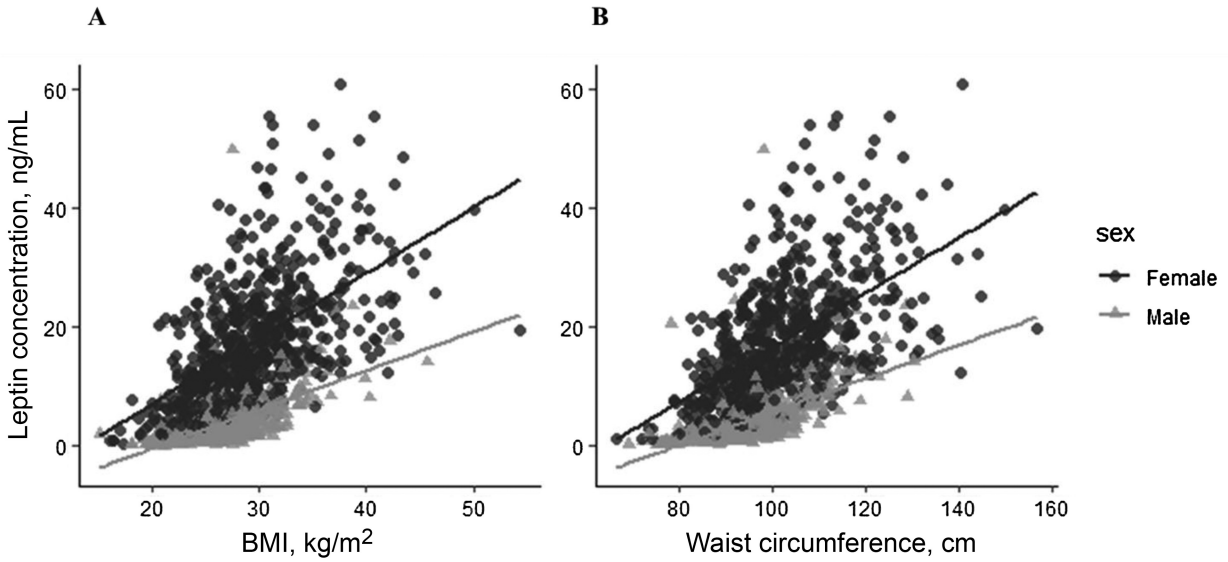

FIGURE 1 Correlations between fasting leptin concentrations and measurements of overall (A) and central adiposity (B). Spearman's ρ for leptin and BMI ($n = 1112$): women = 0.6, men = 0.7. Spearman's ρ for leptin and waist circumference ($n = 1112$): women = 0.6, men = 0.8. Fitted lines were based on simple linear regression models.

Following previously described modeling strategies, we constructed difference-in-difference models to investigate the intention-to-treat impact of exposure to *atole* in the full first 1000 d compared with partial or no exposure on cardiometabolic disease risk factors (9). The primary outcome variables were fasting glucose, HOMA-IR, HOMA-B, and leptin concentration, and the secondary variables were BMI, waist circumference, percentage body fat, and fasting insulin concentration. Because there are multiple sibling sets in our data, we controlled for clustering at the household level by generating cluster-robust estimates of the variance matrix. We built a series of models. Our base model (Model 1) included 3 independent variables: *1*) the treatment variable (receiving either *atole* or *fresco* during the nutritional supplementation trial)—because the randomization was at the village level, we used birth village in place of the binary "*atole* compared with *fresco*" variable to control for village-level random effects (controlling for differences between the villages at baseline); *2*) timing of exposure (exposed to either *atole* or *fresco* during the full first 1000 d compared with otherwise); and *3*) the interaction term between treatment and timing of exposure, which is our target difference-in-difference exposure variable. This variable represented participants who were exposed to *atole* during the full first 1000 d compared with those with partial or no exposure in this timeframe. We controlled for birth year and sex in the base models. When biomarkers and T2DM were the dependent variables in the base models, we also adjusted for BMI and waist-to-height ratio.

The difference-in-difference approach controls for within-village fixed effects that might otherwise differ between individuals. However, there were still potential between-group differences using this approach. We therefore built adjusted models that sequentially added childhood characteristics [socioeconomic status, (SES), tertiles in early life, maternal age at childbirth, maternal height, and maternal schooling—Model 2], adulthood characteristics (SES in 2015, grades of schooling completed by the participant, and residence in Guatemala City—Model 3), and adiposity measurements (BMI for total adiposity and waist-to-height ratio for central distribution of adiposity—Model 4). We presented Model 3 (for anthropometric measurements as dependent variables) and Model 4 (for biomarkers as dependent variables) as the adjusted models. For pooled models, we assessed stratum heterogeneity by sex through testing an interaction term between sex and the difference-in-difference exposure variable. Even when stratum heterogeneity was not detected, we also conducted sex-specific analysis owing to the biological differences between the 2 sexes, especially because of the significantly higher leptin concentration in women than in men.

We conducted sex-specific mediation analysis to investigate the role of leptin in the difference-in-difference models. We used the Baron and Kenny method (25). (Refer to Figure 2 in the Results section for annotations.) The direct model included the glycemic measurements (fasting glucose, HOMA-IR, and HOMA-B, respectively) as the outcome and the difference-in-difference exposure variable as the predictor [glycemic measurement = $\hat{c} \times$ (exposure to *atole* in the full first 1000 d) + (control variables) + ε_1]. The standardized regression coefficient of the exposure variable was the total effect $\hat{c}$. The mediation model had the same outcome and predictor with leptin being added as a mediator [glycemic measurement = $\hat{c}' \times$ (exposure to *atole* in the full first 1000 d) + $\hat{b} \times$ (fasting leptin) + (control variables) + ε_2]. Then, treating leptin as the outcome, the exposure variable had a coefficient $\hat{a}$ [leptin = $\hat{a} \times$ (exposure to *atole* in the full first 1000 d) + (control variables) + ε_3]. The mediation effect was the product of $\hat{a}$ and $\hat{b}$, which represents the indirect pathway between exposure and outcome. The mediation percentage was the indirect effect $\hat{a}\hat{b}$ divided by the total effect $\hat{c}$. When the indirect pathway $\hat{a}\hat{b}$ suppressed the total direct effect $\hat{c}$ (e.g., the sign flipped), we did not report the mediation percentage. Control variables were included as in the unmediated difference-in-difference models. We ruled out

TABLE 2 Difference-in-difference estimates for exposure to *atole* during the full first 1000 d compared with partial or no exposure in predicting cardiometabolic risk factors[1]

Characteristics	Women β (95% CI)	Men β (95% CI)	Pooled β (95% CI)
Anthropometry			
BMI, kg/m^2			
Base	1.0 (–0.4, 2.5)	1.4 (–0.1, 2.9)	1.2 (0.1, 2.2)
Adjusted	0.9 (–0.6, 2.4)	1.7 (0.3, 3.1)*	1.3 (0.2, 2.3)*
Waist circumference, cm			
Base	1.1 (–2.5, 4.6)	4.6 (1.1, 8.0)*	2.3 (–0.2, 4.9)
Adjusted	1.0 (–2.6, 4.6)	5.2 (2.0, 8.5)**	2.7 (0.1, 5.2)
Body fat percentage			
Base	0.8 (–0.9, 2.5)	2.3 (–0.2, 4.7)	1.3 (–0.2, 2.8)
Adjusted	0.7 (–1.0, 2.4)	2.8 (0.4, 5.1)*	1.4 (–0.03, 2.9)
Biomarkers			
Fasting insulin, mIU/L			
Base	0.8 (–2.8, 4.4)	–1.5 (–4.4, 1.3)	–0.2 (–2.8, 2.4)
Adjusted	0.7 (–3.1, 4.5)	–0.8 (–3.7, 2.2)	–0.02 (–2.8, 2.7)
Fasting glucose, mmol/L			
Base	–0.8 (–1.7, 0.1)	0.2 (–0.6, 0.9)	–0.4 (–1.1, 0.2)
Adjusted	–0.8 (–1.8, 0.2)	0.2 (–0.5, 0.9)	–0.4 (–1.0, 0.3)
HOMA-IR			
Base	–0.2 (–1.3, 0.8)	0.2 (–0.9, 1.3)	–0.1 (–0.9, 0.7)
Adjusted	–0.5 (–1.5, 0.6)	–0.1 (–1.0, 0.8)	–0.3 (–1.1, 0.4)
HOMA-B			
Base	4.5 (–26.6, 35.6)	0.3 (–34.4, 35.1)	1.5 (–22.5, 25.5)
Adjusted	–7.6 (–36.6, 21.4)	–10.2 (–39.5, 19.2)	–9.0 (–30.8, 12.7)
Fasting leptin, ng/mL			
Base	2.6 (0.2, 5.1)*	–0.8 (–2.3, 0.6)	1.2 (–0.4, 2.8)
Adjusted	2.8 (0.3, 5.3)*	–0.7 (–2.1, 0.8)	1.2 (–0.4, 2.8)

[1]For anthropometric measurements each as a dependent variable, the base models were as follows: anthropometry = birth villages + timing of exposure + (*atole* compared with *fresco*) × (timing of exposure) + birth year + sex. The adjusted models controlled for childhood characteristics (childhood SES tertiles dummy variables, maternal age at childbirth, maternal height, and maternal schooling) and adult characteristics (2015 SES tertiles dummy variables, grades of schooling completed, and Guatemala City residence). The coefficients presented were for the interaction term (*atole* compared with *fresco*) × (timing of exposure). For biomarkers each as a dependent variable, the base models were as follows: biomarker = birth villages + timing of exposure + (*atole* compared with *fresco*) × (timing of exposure) + birth year + sex. The adjusted models controlled for childhood characteristics (childhood SES tertiles dummy variables, maternal age at childbirth, maternal height, and maternal schooling), adult characteristics (2015 SES tertiles dummy variables, grades of schooling completed, and Guatemala City residence), and measurements of overall and central adiposity (BMI and waist-to-height ratio). The coefficients presented were for the interaction term (*atole* compared with *fresco*) × (timing of exposure). For pooled models, we tested stratum heterogeneity by sex through constructing the interaction term between sex and the difference-in-difference exposure variable. None of these tests had a P value < 0.05. We did not adjust for sex in sex-specific models. *,**Significant difference: $^*P < 0.05$, $^{**}P < 0.01$. HOMA, homeostasis model assessment; HOMA-B, HOMA of pancreatic β-cell function; HOMA-IR, HOMA of insulin resistance; SES, socioeconomic status.

moderated mediation by testing the potential moderating effect by leptin through adding an interaction term between leptin and the exposure variable. We confirmed the results through simulation exercises using the statistical package "mediation" in R, bootstrapped 1000 times (25, 26). We used the "RMediation" package to obtain the 95% CI of the mediation effect (27).

We also conducted a sensitivity analysis to account for a potential hormonal impact on biomarker concentrations: we compared the mediation results between postmenopausal women and other women. We categorized women who did not have menstruation for ≥12 consecutive months at the time of data collection as postmenopausal (28).

We conducted all analyses in R version 3.6.0 (R Core Team 2018, Foundation for Statistical Computing). Statistical significance was set a priori at P value < 0.05. All P values were 2-sided.

Research ethics

The study was approved by the Institutional Review Board at Emory University and the Ethics Review Committee of INCAP. All study participants provided written informed consent in Spanish.

Results

The sample included 1112 Guatemalan adults (60.6% women) with a mean ± SD age of 44.2 y ± 4.3 y for women and 43.9 y

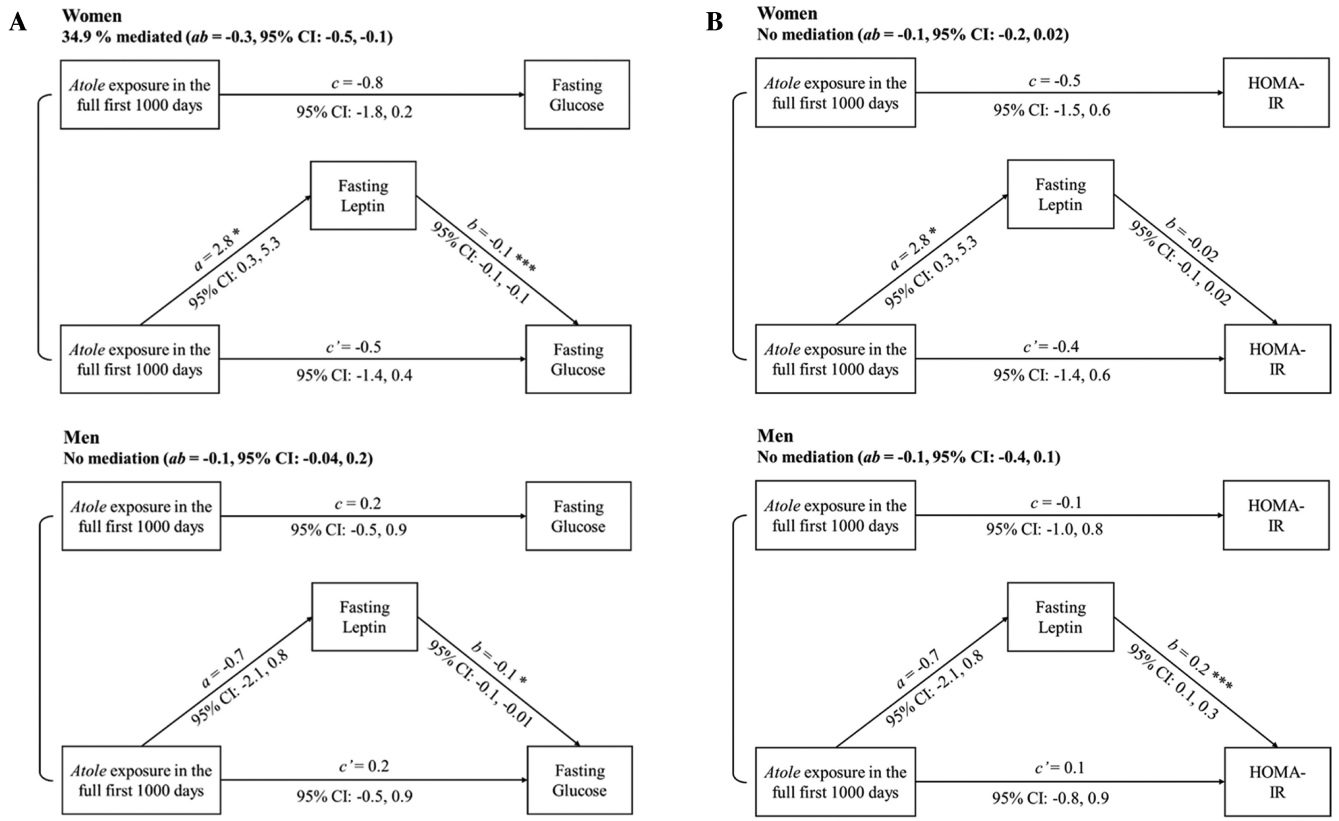

FIGURE 2 Mediation analysis of leptin in the pathway between exposure to *atole* in the full first 1000 d and 3 glycemic measurements: fasting glucose (A), HOMA-IR (B), and HOMA-B (C). Total effect $c = c' + ab$; mediation effect $= ab$; percentage mediated $= ab/c \times 100\%$. Direct model: glycemic measurements $= c \times$ (exposure to *atole* in the full first 1000 d) + (control variables) + ε. Mediation model: *1)* glycemic measurements $= c' \times$ (exposure to *atole* in the full first 1000 d) + $b \times$ (fasting leptin) + (control variables) + ε'; *2)* leptin $= a \times$ (exposure to *atole* in the full first 1000 d) + (control variables) + ε". Control variables included birth villages, timing of exposure, birth year, childhood characteristics (SES in childhood, maternal age at childbirth, maternal height, and maternal schooling), adult characteristics (SES in 2015, grades of schooling completed, Guatemala City residence), and anthropometry (BMI and waist-to-height ratio). *,***Significant associations: $^{*}P < 0.05$, $^{***}P < 0.001$. HOMA-B, HOMA for β-cell function; SES, socioeconomic status.

± 4.1 y for men (**Table 1**). Approximately 1 in 5 participants were exposed to *atole* during the full first 1000 d. Men and women were similar in terms of most sociodemographic factors investigated. Women had higher BMI, waist circumference, and percentage body fat than men (Table 1). Based on BMI, ∼40% of women and 20% of men were obese. Based on sex-specific waist circumference standards, almost 90% of women and 20% of men were centrally obese. Over 30% of all participants were hyperglycemic. More women than men had T2DM (16.5% and 9.2%, respectively), and more women than men (<10% for both) were taking medications to manage their diabetic condition (Table 1). Compared with men, women had higher fasting concentrations of insulin, glucose, and leptin, and higher HOMA-IR and HOMA-B. Leptin concentration was positively correlated with both BMI (Spearman's ρ was 0.6 for women and 0.7 for men) and waist circumference (Spearman's ρ was 0.6 for women and 0.8 for men) (**Figure 1**).

Based on results from the adjusted models, the mean concentration of glucose was 0.8 mmol/L lower (95% CI: –1.8, 0.2 mmol/L) in women who were exposed to *atole* in early life than in unexposed women. Among women, leptin concentration was 2.8 ng/mL higher (95% CI: 0.3, 5.3 ng/mL) and HOMA-IR was 0.5 lower (95% CI: –1.5, 0.6) in the exposed group than in the unexposed group (**Table 2**). Among men, being in the exposure group was associated with lower leptin concentration (–0.7 ng/mL; 95% CI: –2.1, 0.8 ng/mL) and higher fasting glucose concentration (0.2 mmol/L; 95% CI: –0.5, 0.9 mmol/L). Exposure to *atole* during the first 1000 d was positively associated with a few measurements of fatness (Table 2). In the pooled analysis, exposure group was associated with a BMI higher by 1.3 (95% CI: 0.2, 2.3) and a 2.7-cm (95% CI: 0.1, 5.2 cm) larger waist circumference. Among men, exposure group was associated with a BMI higher by 1.7 (95% CI: 0.3, 3.1), a 5.2-cm (95% CI: 2.0, 8.5 cm) larger waist circumference, and a 2.8% increase in body fat percentage (95% CI: 0.4%, 5.1%). We did not observe significant stratum heterogeneity by sex in the pooled models.

Among women, leptin mediated the pathway between early-life *atole* exposure and fasting glucose concentration (**Figure 2**). In adjusted models for women, leptin mediated 34.9% of the nutrition exposure–glucose association (mediation effect = –0.3 mmol/L; 95% CI: –0.5, –0.1 mmol/L) (Figure 2A). Leptin did not mediate the pathway between early-life nutritional exposure and HOMA-IR in women (Figure 2B), but it did mediate the pathway to HOMA-B: the indirect effect through leptin was 8.1 mmol/L (95% CI: 1.8, 14.9 mmol/L) and the direct effect

in the mediation model was –7.6 mmol/L (95% CI: –36.6, 21.4 mmol/L) (Figure 2C). We did not observe any mediation effect of leptin on fasting glucose, HOMA-IR, or HOMA-B in men (Figure 2A–C). We confirmed the results of the mediation analysis by bootstrapped simulation: as shown in **Supplemental Figure 2**, the average causal mediation effect was consistent in bootstrapped simulation results for all 3 glycemic measurements by sex.

In sensitivity analyses, mediation analysis showed a significant mediating effect of leptin between early-life *atole* exposure and fasting glucose concentration in all other women (38.7% mediated), but not in postmenopausal women. Nevertheless, the coefficients (*a*, *b*, *c*, and *c'*) in the 2 sets of mediation models were similar between postmenopausal women and other women (**Supplemental Figure 3**).

Discussion

To our knowledge, this study is among the first to use biomarker data at the population level to investigate potential biochemical mechanisms through which early-life nutritional exposure can have long-term cardiometabolic impacts. Previous articles from the INCAP study have documented associations between early-life exposure to *atole* and positive health and human capital outcomes (1). In the current article, we showed that leptin partially mediated the association of early-life nutritional exposure and glycemic measurements in women. We did not observe the same mediation effect in men. The mediation in women was mainly driven by improved pancreatic β-cell function (leptin was associated with an increase in HOMA-B via the indirect pathway in mediation analysis), and not by a reduction in insulin resistance (there was no mediation for HOMA-IR). We confirmed that the protein- and energy-containing nutritional supplement *atole* had mixed effects on long-term cardiometabolic risk factors: early-life exposure to *atole* was associated with lower fasting glucose concentrations (in women). The same exposure, however, was also associated with increased odds for overall and central adiposity (mainly in pooled models). As hypothesized, we observed a positive correlation between leptin and adiposity measurements in both sexes, both overall adiposity and central adiposity. We also observed significantly higher leptin concentrations in women than in men.

This set of analyses was guided by a conceptual framework to draw linkages between early-life nutrition, human ontogeny, and relevant cardiometabolic pathways, emphasizing the role of leptin (**Figure 3**). Previous research suggested that early-life exposure to improved nutrition affects the development of metabolically active tissues, including adipose tissue, skeletal muscle, pancreas, liver, and the brain (6, 7, 29). Nutritional exposure influences leptin concentration through adipose tissue and other pathways, including nutrition signaling, hormonal regulation, and psychoneurological regulation (30). Measurements of overall and central adiposity in adulthood reflected 2 different sources of adiposity: adipose tissue influenced by early-life nutritional exposure (cell size, depot, and the type of adipose tissue), and increase in adiposity due to an obesogenic environment. All of these factors predicted obesity risk and determined circulating leptin concentrations (30, 31). Conversely, increased leptin concentration may also increase later adiposity through the leptin-dependent feedback loop, especially when there is physiologic leptin insensitivity (12). Indeed, we observed a positive association between leptin concentration and measurements of overall and central adiposity for both men and women.

As a catabolic hormone, leptin plays a central role in glucose regulation: it promotes glucose uptake by skeletal muscle and other peripheral tissues, reduces hepatic gluconeogenesis, and has direct effects on the central nervous system (14–16). Researchers have found that early-life exposure to improved nutrition can help guide more stem cells to prioritize myogenesis over adipogenesis, which predetermines adulthood muscle mass and intramuscular fat content (32). Improved nutritional exposure in early life also supports the development of hepatic tissue, which is central to gluconeogenesis and glucose storage. Brain and other peripheral tissues that actively utilize glucose also benefit from early-life exposure to improved nutrition. This is consistent with earlier reports of a strong association between *atole* supplementation and increased lean mass and larger head circumference in the INCAP population (33, 34). Our current data suggest that exposure to *atole* in early life may have positively affected tissue development in this chronically malnourished population by providing the basis for leptin to exert euglycemic regulation.

Leptin also participates in glucose homeostasis through its effects—both acute and chronic—on the pancreas (17, 35, 36). Leptin can lower glucose concentration through inhibiting

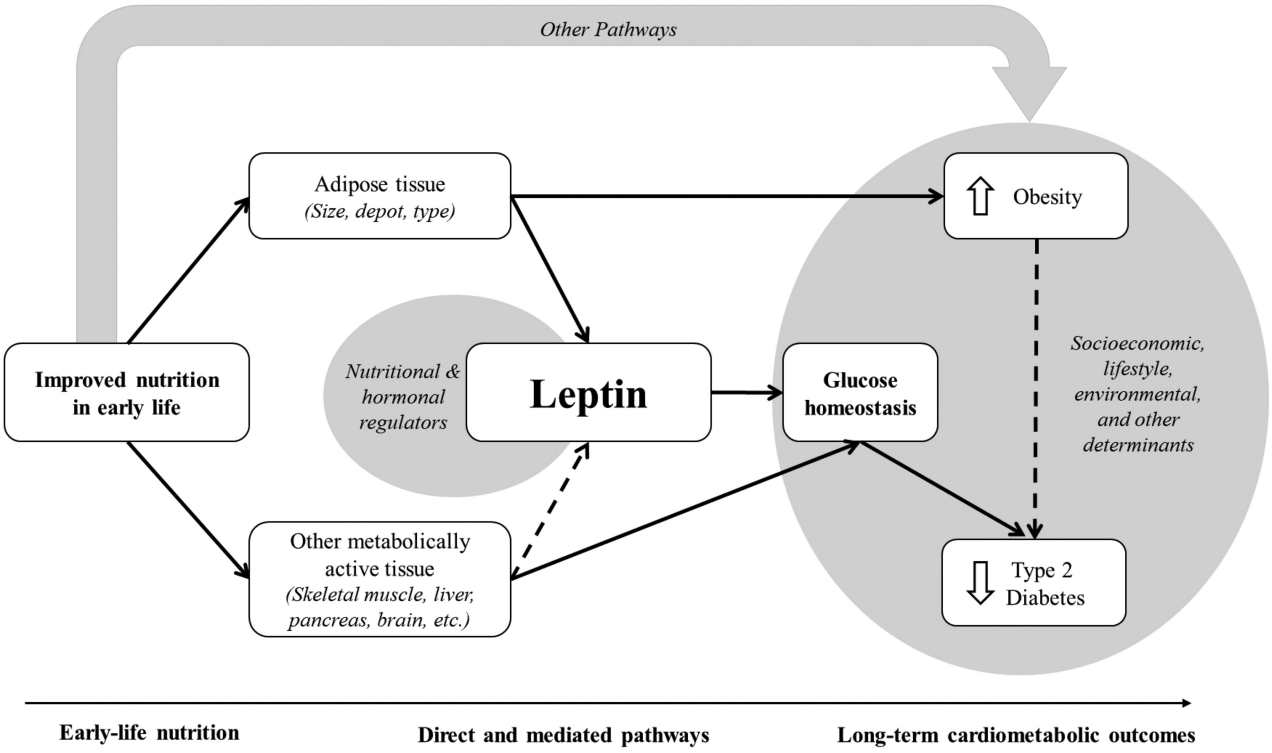

FIGURE 3 Conceptual framework centering leptin in pathways between early nutrition and long-term cardiometabolic outcomes. The conceptual framework mapped out simplified pathways between exposure to improved nutrition in early life and the ontogenic effects on metabolically active tissues, including adipose tissue, hepatic tissue, pancreas, skeletal muscle, and brain tissue. Leptin is proportional to adipose tissue mass. Leptin is a key glucose-lowering agent in this conceptual framework, which helped elucidate the differential associations between early nutrition and 2 cardiometabolic outcomes: obesity and type 2 diabetes. Other nonbiological determinants are summarized in the shaded ovals, following the gray shaded arrow, but were not the focus of our analysis.

glucagon release from pancreatic α-cells, countering its glucose-raising effect (17). Pancreatic β-cells are sensitive to maternal diet and the in-utero nutritional environment and can play an important role in insulin secretion (37). Insulin can, in turn, chronically upregulate both the production and secretion of leptin (38). However, in obese individuals with chronic leptin resistance, leptin can impair pancreatic β-cell function and disrupt insulin secretion (36). In this study we observed a positive association between leptin and HOMA-B, suggesting that this population may not be leptin-resistant despite the high prevalence of obesity.

Consistent with a recent meta-analysis that reported a difference in the association of leptin and diabetes between men and women, we also noted that leptin had sex-specific mediation effects in our study (39). Previous research indicated that, owing to differences in metabolic programming, women are predisposed to obesity and metabolic syndrome and men to diabetes—although this difference was not observed in our study (40). Sensitivity analysis between postmenopausal women and all other women indicated that the observed sex-specific differences may not be explained merely by hormonal differences. Although the leptin pathway is partially explanatory in the sex-specific differences, other factors may play a role as well, including the potential impact of early-life nutrition on myogenesis and adipogenesis, which contribute to differences in body composition between men and women. Adiposity is not only affected by early-life nutritional status, but also by external factors such as occupational, environmental, and lifestyle differences between the 2 sexes (40, 41). Men are engaged in more manual work and physical activity than women in this study population. Long-term improvements in work capacity and wages were also documented among men who received improved nutrition in early childhood in this population (1). These factors can affect adiposity, circulating leptin concentration, and glycemic status.

Our observation that leptin statistically mediated the pathways between early nutrition and long-term glycemic status supports the biological postulations. Nevertheless, population-level data have not shown a consistent association between leptin and diabetes or fasting glucose concentration: leptin was used in animal models to reverse diabetes, but epidemiological data in human populations mainly reported a null to positive correlation between leptin and diabetes, with only a few exceptions (39, 42–45). It is possible that the participants who were exposed to *atole* in early life did not develop resistance to leptin even when their risk of obesity increased, thus allowing leptin to perform its expected catabolic functions. This postulation warrants further investigation.

There are a few limitations to our current analysis. First, there was missingness in several confounding factors. We used multiple imputation methods to attenuate any potential bias. Second, the biomarker data reflect a cumulative effect of early-life nutritional exposure and ensuing lifestyle and environmental

factors over a span of close to 50 y. We do not have ontogeny information and all relevant exposure data throughout the life course to confirm several assumptions made in the conceptual framework, but we have reviewed literature in animal models to help discuss the biological plausibility. In addition, although the "first 1000 days" is an important concept, the actual developmental processes do not follow this exact timeframe. Lastly, our study population included only Guatemalan adults within a relatively narrow age range (born during 1962–1977). When considering the generalizability of our study, findings from other similar studies should be taken into consideration to properly interpret the results.

At a mean age of 44 y, both men and women in this Guatemalan cohort had high prevalence of cardiometabolic conditions. We identified a positive association between leptin and body adiposity in both sexes, as well as the mediation effect of leptin on long-term glucose regulation among female participants. The underlying reasons for the observed sex-specific differences in the leptin mediation effect should be further investigated.

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