International Journal of Obesity www.nature.com/ijo

ARTICLE

Clinical Research

Supplementation with antioxidant micronutrients in pregnant women with obesity: a randomized controlled trial

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BACKGROUND/OBJECTIVE: Obesity increases maternal morbidity and adversely affects child health. Maternal inflammation may play a role in adverse outcomes. The objective of this study was to determine whether providing a higher dose of antioxidant micronutrients to pregnant women with obesity would raise concentrations of key antioxidant vitamins and impact inflammation and oxidative stress during pregnancy.
SUBJECTS/METHODS: This was a double-blind, randomized controlled trial. We recruited pregnant women with a body mass index (BMI) ≥ 30 kg/m^2 at their initial prenatal visit (< 13 weeks gestation) and collected blood and urine samples at baseline, 24–28 weeks, and 32–36 weeks to measure micronutrient concentrations (vitamin C, E, B_6 and folate), markers of inflammation (C-reactive protein, interleukin-6, 8, and 1β) and oxidative stress (8-epi-PGF2α and malondialdehyde). We collected maternal and infant health data from enrollment to delivery as secondary outcomes. We enrolled 128 participants (64 in each arm), and 98 (49 in each arm) completed follow-up through delivery.
INTERVENTION: Both groups received a standard prenatal vitamin containing the recommended daily allowance of micronutrients in pregnancy. In addition, the intervention group received a supplement with 90 mg vitamin C, 30 αTU vitamin E, 18 mg vitamin B_6, and 800 µg folic acid, and the control group received a placebo.
RESULTS: The intervention group had higher vit B_6 (log transformed (ln), β 24–28 weeks: 0.76 nmol/L (95% CI: 0.40, 1.12); β 32–36 weeks: 0.52 nmol/L (95% CI: 0.17, 0.88)) than the control group. Vitamins C, E, erythrocyte RBC folate concentrations did not differ by randomization group. The intervention did not impact biomarkers of inflammation or oxidative stress. There were no differences in maternal or neonatal clinical outcomes by randomization group.
CONCLUSIONS: Higher concentrations of antioxidant vitamins during pregnancy increased specific micronutrients and did not impact maternal inflammation and oxidative stress, which may be related to dosing or type of supplementation provided.
CLINICAL TRIAL REGISTRATION: Clinical Trial Identification Number: NCT02802566; URL of the Registration Site: www.clinicaltrials.gov.

International Journal of Obesity; https://doi.org/10.1038/s41366-024-01472-z

INTRODUCTION

Over half of the reproductive age women in the US are overweight or have obesity [1]. Obesity increases pregnancy-related maternal morbidity and adversely affects child health throughout the lifespan [2, 3]. In the long-term, infants of women with obesity are 2–3 times more likely to develop obesity and metabolic syndrome themselves [4], thus perpetuating this cycle.

Convincing evidence supports the pathogenic role of in utero dysmetabolism in intergenerational obesity. The in utero environment in a pregnancy affected by obesity is characterized by metabolic dysregulation, including insulin resistance, hyperlipidemia, vascular dysfunction, inflammation, and oxidative stress [5]. Both intrinsic (e.g., adipose tissue, vascular dysfunction) and extrinsic (e.g., diet, stress) sources of inflammation likely contribute to this milieu [6]. In a healthy pregnancy, inflammation is carefully orchestrated throughout gestation to support implantation, placentation, fetal growth, and parturition, ensuring offspring survival. Obesity is characterized by chronic, metabolically induced inflammation. Numerous studies have reported that pregnant women with obesity have a dysregulated inflammatory profile, with some inflammatory markers increased, peaking in the second trimester, compared to healthy, lean pregnant women [7–12].

We have previously demonstrated the importance of oxidative stress in the pathogenesis of intergenerational obesity in an

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Received: 3 October 2023 Revised: 3 January 2024 Accepted: 15 January 2024
Published online: 23 February 2024

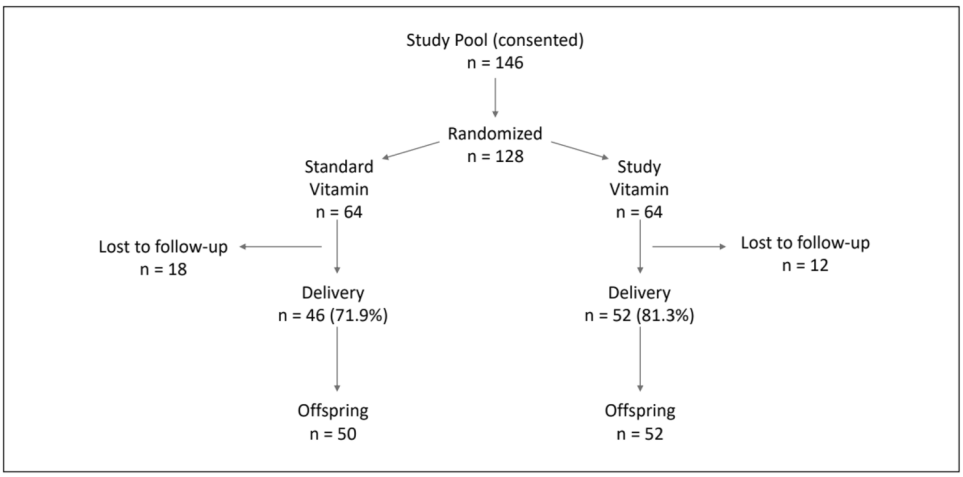

Fig. 1 BMI-based Prenatal Vitamin CONSORT flow diagram.

animal model [13]. Pups born to obese rat dams who were supplemented with an antioxidant cocktail before and during pregnancy had lower adiposity and improved glucose tolerance, inflammation, and oxidative stress. In addition, we and others have reported that systemic concentrations of antioxidant micronutrients vitamins B_6 [14], B_{12} [15–17], C [18], E [19], and folate [14–21] are lower in pregnant women with obesity compared to non-obese pregnant women, and that concentrations of these circulating micronutrients are inversely associated with inflammation and oxidative stress. However, there is a gap in our understanding of the causal pathways linking maternal antioxidant micronutrient concentrations to inflammation and oxidative stress in pregnancies complicated by obesity.

Based on these observations and the gaps in the literature, the objective of this trial was to test if the pro-inflammatory, pro-oxidant milieu that accompanies maternal obesity can be ameliorated by supplementing pregnant women with obesity with specific antioxidant micronutrients. We hypothesized that providing higher concentrations of antioxidant micronutrients than those provided in standard prenatal vitamin and mineral supplements would raise systemic concentrations of these micronutrients (primary outcome) and decrease markers of inflammation and oxidative stress (secondary outcome) compared to those receiving standard supplementation. We also explored how this intervention impacted maternal, perinatal and infant outcomes.

MATERIALS/SUBJECTS AND METHODS

Study design

This was a double-blind, randomized controlled trial (RCT). The composition of the vitamins was determined based on preliminary studies reporting lower concentrations of vitamin C, E, B_6, and folate in women with higher BMI in mid-gestation, with dosages determined using pharmacokinetic modeling assuming a one-compartment model [18]. In a formative pilot cohort in Boston, MA, we demonstrated that pregnant women with obesity had significantly lower levels of vitamins B_6, C, E, and red blood cell folate specifically, but not other measured antioxidant micronutrients such as vitamin D, A, B_{12}, or zinc. Given the importance of demographic and population characteristics such as diet in micronutrient concentrations, we utilized this data as the basis for our intervention. We modeled the doses for this trial using a one-compartment pharmacologic model with linear regression, estimating the dosing required to replete systemic concentrations from women with obesity (mean BMI of 35 kg/m^2) to the mean concentrations in a lean population (mean BMI 22.5 kg/m^2).

Both groups received a standard prenatal vitamin containing the recommended daily allowance of micronutrients in pregnancy (Supplementary Table 1). In addition, the intervention group received a supplement with 90 mg vitamin C as ascorbic acid, 30 αTU vitamin E as d-α tocopherol acetate, 18 mg of vitamin B6 as pyridoxine hydrochloride, and 800 µg folic acid, and the control group received a placebo composed of lactose, maltodextrin, and cellulose that was identical in appearance. All supplements were provided by Tischcon Corp (Westbury, NY.)

Subjects (Fig. 1)

We recruited pregnant women with a BMI ≥ 30 kg/m^2 at their initial prenatal visit (< 13 weeks gestation) at two academic medical centers: Brigham and Women's Hospital (BWH) and Beth Israel Deaconess Medical Center (BIDMC), both in Boston, MA. Exclusion criteria were: autoimmune disease, stage two or three hypertension, history of smoking cigarettes within the past year, history of an infant with a congenital anomaly, recurrent pregnancy loss, vegan diet, eating disorder, conception with in vitro fertilization, or lactose intolerance (given the composition of the placebo). At the recruitment visit, study staff obtained written informed consent and subjects were randomized using random permuted blocks of four in a 1:1 ratio to the intervention or control group. The research pharmacies dispensed a standard prenatal vitamin for all subjects and either a micronutrient supplement (intervention) or a placebo (control). Phlebotomists collected blood and subjects provided urine samples at < 13 weeks, 24–28 weeks, and 32–36 weeks. Participants self-reported demographics through questionnaires at recruitment. Research staff counted remaining study supplements at each study visit to assess compliance. Participants self-reported adverse effects to study staff. Both participants and research staff were blinded to group assignment. All research staff remained blinded until after the last 12-month infant follow-up visit. This study was approved and supervised by the BWH (Partners) Institutional Review Board and safety was monitored by an independent medical monitor.

Laboratory assays

At < 13 weeks, 24–28 weeks and 32–36 weeks of gestation we measured micronutrient concentrations and markers of inflammation and oxidative stress. *Micronutrients:* EDTA plasma samples for Vitamin C were treated within minutes of collection with perchloric acid to avoid oxidation. The protein free supernatant was measured for vitamin C by High Performance Liquid Chromatography (HPLC) (Waters HPLC Empower® Network system with the 717plus autosampler, 515 pump, Waters Corporation, Milford, MA

01757) using electrochemical detector (BAS EC-5, Bioanalytical Systems Inc., West Lafayette, IN 47906) according to Behrens et al. The intra- and inter- assay coefficients of variability (CVs) are 4.5% and 6.5%, respectively accordingly [22]. Erythrocyte folate was measured by a chemiluminescent, competitive immunometric assay, (IMMULITE 2000, Siemens Healthcare Diagnostics, Los Angeles, CA 90045). The intra- and inter- assay CVs are 7.5% and 9.0%, respectively accordingly (https://www.scribd.com/document/475502808/the-immunoassay-handbook-immulite-and-immulite-1000). Pyridoxal 5'-phosphate (Vitamin B6) in plasma was measured by a radio-enzymatic immunoassay using a Packard Tri-Carb 2100TR liquid Scintillation Counter, (Thermo Fisher Scientific, Waltham, MA 02454) according to Camp et al. The intra- and inter- assay CVs are 5% and 7%, respectively accordingly [23]. *Markers of oxidative stress:* Malondialdehyde (MDA) in plasma was measured by HPLC (Aligent 1100 HPLC System, Santa Clara, CA) with fluorometric detection according to Behrens et al. with some modifications. The intra-day and inter-day coefficient of variation (CV) are smaller than 5% [24]. 15-Isoprostane $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) in urine was measured by a competitive enzyme-linked immunoassay (Eagles Biosciences, Inc., Nashua, NH 03063) after treatment with an enhanced dilution buffer that eliminates interference due to non-specific binding. The inter-assay CV was less than 10%. Urine creatinine was measured using standard laboratory methods and 8-epi-$PGF_{2\alpha}$ to creatinine ratio calculated. *Markers of inflammation:* CRP, IL-6, IL-8, IL-1β: IL-6 (low limit of detection IL-6 0.105 pg/ml), IL-8 0.040 pg/ml, IL-1β 0.019 pg/ml, CRP 1.33 pg/mL) were measured by a Meso Scale Discovery (Gaithersburg, MD) electrochemiluminescence multiplex platform. All samples were tested in duplicate.

Clinical data

Maternal Data: Study staff collected data from the electronic health record on the clinical variables at each study visit (Table S2) and during the delivery/neonatal hospitalization.

Research measures

Between 24 and 48 h after delivery, trained research staff measured infant weight on a calibrated scale, length on a recumbent stadiometer, waist circumference with a paper tape measure, adiposity with air displacement plethysmography (Peapod^{TM}), and skin fold thickness (SFT) with the Harpenden caliper (bicep, tricep, suprailiac, and subscapular). All study measures were conducted in duplicate and a third measure repeated if the initial two readings differed by a predetermined amount (weight 10 g, length 0.1 cm, SFT 0.1 cm).

Sample Size calculation

Based on our preliminary data, we performed a power calculation with the 4 primary outcomes of concentrations of vitamins C, E, B_6, and erythrocyte folate equally weighted ($\alpha = 0.0125$ given multiple comparisons). The standard deviations (SDs) used in the calculations were 0.3 mg/dL for C, 32 ug/dL for E, 50 mM/L for B_6, and 500 ng/ml for folate. Thus, a sample size of 100 ($n = 50$ in each group) was predetermined to have 80% power to observe a difference of at least 0.19 mg/dL, 20 ug/dL, 31 mM/L, and 309 ng/ml for C, E, B_6, and erythrocyte folate, respectively.

Statistical methods

Population characteristics: We report descriptive statistics of baseline demographic and clinical characteristics overall and by treatment arm for the total, randomized sample ($n = 128$). *Outcomes:* We evaluated the effect of intervention on outcome using intention-to-treat (ITT), in accordance with CONSORT guidelines [25], followed by per protocol analysis. ITT analysis was modeled using linear regression with missing data imputed using multiple imputation by chained equations (MICE) and predictive mean modeling under the assumption of data missing at random (MAR). Analyses were performed using models both unadjusted and adjusted for imbalance in a priori baseline covariates prognostic of outcome (maternal educational attainment, and baseline serum measurement, infections, prenatal vitamins, and analgesic medication use) [26–29]. Outcomes were natural log (ln) transformed to meet linear regression normality assumptions except vitamin C which met regression assumptions without transformation. The α was adjusted for the co-primary outcomes at two timepoints. Changes over time were analyzed by group with paired t-tests. *Clinical outcomes (secondary analysis):* We used median and logistic regression to quantify the association between treatment arm and measures of pregnancy and birth outcomes, respectively. Analyses of infant outcomes were adjusted for intrafamilial correlation among twins using clustered standard errors (median regression) or generalized estimating equations (logistic regression) using an exchangeable covariance structure. We further sought to quantify how the cumulative exposure to systemic nutrients or biomarkers during the intervention period was associated with pregnancy and birth outcomes by calculating the total area under the curve (AUC) for the 3 different timepoints of measurement for each nutrient or biomarker. We then dichotomized the AUC as > 75th percentile versus <= 75th percentile and examined the association of this exposure measure with the clinical outcomes using median regression for continuous and logistic regression for dichotomous outcomes. Analyses were run using SAS v9.4 software (SAS Institute, Cary, NC) with the exception of median regression analyses performed using STATA software and the qreg wrapper qreg2 for clustered standard errors (Stata v17.0, StatCorp, College Station, TX).

RESULTS

Baseline participant characteristics, safety and compliance

As noted in Table 1, the median (IQR) BMI was 33.5 (31.6, 38.6) kg/m^2 in the intervention group and 34.6 (31.5, 39.0) kg/m^2 in the control group. The study population had a higher proportion of Black and Hispanic subjects and lower education and household income than the overall Boston, MA population, but reflected the demographics of the pregnant population with obesity at BWH and BIDMC [30]. Baseline demographic and clinical covariates were generally balanced between treatment arms; however, the control group was less likely to have a college degree (control: 35.9% vs. intervention 54.7%), had a higher rate of sexually transmitted infections (control: 18.8% vs. intervention 6.3%), and was more likely to be receiving antimicrobials (control: 14.1% vs. intervention 4.7%) or analgesics (control: 7.8% vs. intervention 0%). Reported adverse events did not differ by treatment group and were predominantly limited to minor and self-resolving gastrointestinal symptoms. Only one adverse event of hospitalization due to transaminitis and abdominal pain was reported during the trial in the control group, which was adjudicated by the independent medical monitor to be unrelated to the study intervention. Compliance to study intervention did not differ by intervention group (Table 1). There were no differences in baseline characteristics in subjects who completed the study ($n = 98$) vs. the entire population of recruited subjects ($n = 128$).

Primary outcome

Micronutrient concentrations and markers of inflammation and oxidative stress markers by group are shown in Table 2 and Fig. 2.

By-group comparison at each time point. In the ITT analysis, the intervention led to higher vitamin B_6 concentrations compared to control at 24–28 and 32–36 weeks, in unadjusted and adjusted analyses (adj β 24–28 weeks: 0.76 nmol/L (95% CI 0.40, 1.12) and 32–36 weeks: β 0.52 nmol/L (95% CI 0.17, 0.88)) (Table 2a). Vitamin C, vitamin E, and erythrocyte folate concentrations did not differ

Table 1. Baseline demographic and clinical characteristics among $n = 128$ pregnant women with obesity.

	Total Sample ($n = 128$)		**Study vitamin ($n = 64$)**		**Standard vitamin ($n = 64$)**	
Race (*n*, %)						
White	52	40.6%	25	39.1%	27	42.2%
Black	39	30.5%	22	34.4%	17	26.6%
Asian	2	1.6%	2	3.1%	0	0.0%
Other	12	9.4%	6	9.4%	6	9.4%
N/A	23	18.0%	9	14.1%	14	21.9%
Ethnicity, Hispanic or Latino (*n*, %)						
Hispanic or Latino	48	37.5%	21	32.8%	27	42.2%
Not Hispanic or Latino	76	59.4%	40	62.5%	36	56.3%
Unknown/Not reported	4	3.1%	3	4.7%	1	1.6%
Maternal age (mean, sd)	31	5.6	30.8	5.3	31.3	6.0
Gestational age at study entry (mean, sd)	10.2	1.8	10	1.8	10.4	1.7
BMI (median, IQR)	34.0	31.6, 38.8)	33.5	(31.6, 38.6)	34.6	31.5, 39.0)
BMI category (*n*, %)						
Overweight (BMI 25.0–29.9 kg/m^2)	7	5.5%	2	3.1%	5	7.8%
Obese, Class I (BMI 30.0–34.9 kg/m^2)	65	50.8%	35	54.7%	30	46.9%
Obese, Class II (BMI 35.0–39.9 kg/m^2)	31	24.2%	16	25.0%	15	23.4%
Obese, Class III (BMI ≥ 40 kg/m^2)	25	19.5%	11	17.2%	14	21.9%
Maternal education, college degree or higher (*n*, %)	58	45.3%	35	54.7%	23	35.9%
Annual household income (*n*, %)						
< $20,000	33	25.8%	16	25.0%	17	26.6%
$20,000–$50,000	39	30.5%	18	28.1%	21	32.8%
$50,000–$70,000	14	10.9%	7	10.9%	7	10.9%
$70,000–$100,000	7	5.5%	3	4.7%	4	6.3%
> $100,000	27	21.1%	16	25.0%	11	17.2%
Married (*n*, %)	67	52.3%	37	57.8%	30	46.9%
Primiparous (*n*, %)	41	32.0%	21	32.8%	20	31.3%
Private Insurance (*n*, %)	67	52.3%	36	56.3%	31	48.4%
Medications (*n*, %)						
Currently taking medications (*n*, %)	50	39.1%	20	31.3%	30	46.9%
Antihistamine	20	15.6%	10	15.6%	10	15.6%
Beta-blocker	8	6.3%	6	9.4%	2	3.1%
Contraceptives	14	10.9%	4	6.3%	10	15.6%
Steroids	13	10.2%	8	12.5%	5	7.8%
Asthma medication	19	14.8%	11	17.2%	8	12.5%
Prenatal vitamins	40	31.3%	20	31.3%	20	31.3%
Vitamins, Minerals, Electrolytes	21	16.4%	8	12.5%	13	20.3%
Vitamins with DHA	23	18.0%	14	21.9%	9	14.1%
Antimicrobial	12	9.4%	3	4.7%	9	14.1%
Analgesics	5	3.9%	0	0.0%	5	7.8%
Infections (*n*, %)	18	14.1%	6	9.4%	12	18.8%
Infections, STDs (*n*, %)	16	12.5%	4	6.3%	12	18.8%
Multiple gestation (*n*, %)	4	3.1%	0	0.0%	4	6.3%
Compliance, PNV (*n*, %)						
100%	64	50.0%	34	53.1%	30	46.9%
Compliance, Supplement (micronutrient/placebo), (*n*, %)						
100%	62	48.4%	32	50.0%	30	46.9%

IQR Interquartile range.

Table 2. Maternal Prenatal Serum Markers in the intervention vs. treatment arm among n = 128 participants.

Prenatal Serum Markers	Weeks gestation (GA)	Linear regression effect estimate and CI							
		Unadjusted				Adjusted			
		Estimate	95% CI		*p*-value	Estimate	95% CI		*p*-value
Vitamins									
Vitamin B6, mg									
	< 13	0.07	−0.23	0.37	0.6557	0.06	−0.25	0.37	0.6896
	24–28	0.81	0.46	1.17	< 0.0001*	0.76	0.40	1.12	< 0.0001*
	32–36	0.61	0.26	0.97	0.0008*	0.52	0.17	0.88	0.0042*
Vitamin C, mg									
	< 13	−0.04	−0.18	0.09	0.5169	−0.06	−0.19	0.08	0.4240
	24–28	0.10	−0.04	0.23	0.1553	0.12	−0.01	0.25	0.0669
	32–36	−0.10	−0.24	0.03	0.1364	−0.09	−0.23	0.04	0.1678
Vitamin E, αTU									
	< 13	−0.04	−0.13	0.05	0.4026	−0.06	−0.15	0.04	0.2500
	24–28	0.01	−0.10	0.12	0.8405	0.03	−0.07	0.13	0.5707
	32–36	0.04	−0.08	0.15	0.5144	0.06	−0.05	0.17	0.3156
RBC folate, ng/mL									
	< 13	−0.01	−0.17	0.15	0.9100	−0.03	−0.20	0.13	0.6803
	24–28	0.08	−0.09	0.26	0.3574	0.12	−0.03	0.28	0.1062
	32–36	−0.10	−0.31	0.12	0.3727	−0.06	−0.28	0.15	0.5714
Cytokines and other inflammatory markers									
IL-6, pg/mL									
	< 13	−0.12	−0.36	0.12	0.3324	−0.12	−0.37	0.13	0.3320
	24–28	−0.07	−0.31	0.17	0.5837	0.05	−0.14	0.25	0.5962
	32–36	−0.03	−0.48	0.41	0.8864	0.06	−0.39	0.52	0.7887
IL-8, pg/mL									
	< 13	−0.06	−0.24	0.12	0.5167	−0.07	−0.26	0.12	0.4543
	24–28	−0.02	−0.22	0.18	0.8756	0.02	−0.18	0.22	0.8382
	32–36	0.00	−0.21	0.21	0.9944	0.03	−0.18	0.23	0.7985
CRP, mg/L									
	< 13	−0.29	−0.62	0.04	0.0823	−0.32	−0.66	0.01	0.0551
	24–28	−0.20	−0.50	0.11	0.2051	−0.03	−0.29	0.22	0.7898
	32–36	−0.02	−0.30	0.25	0.8744	0.15	−0.10	0.39	0.2463
Oxidative stress markers									
PGF2α, ng/mg									
	< 13	−0.16	−0.52	0.19	0.3714	−0.14	−0.51	0.22	0.4443
	24–28	0.01	−0.27	0.30	0.9210	0.01	−0.29	0.31	0.9601
	32–36	0.03	−0.20	0.26	0.8081	0.07	−0.16	0.31	0.5486
MDA, uM									
	< 13	0.05	−0.10	0.21	0.4993	0.03	−0.13	0.20	0.6744
	24–28	0.01	−0.15	0.17	0.8911	−0.04	−0.18	0.11	0.6173
	32–36	0.00	−0.17	0.17	0.9736	−0.03	−0.20	0.15	0.7524
Prenatal Serum Markers	**Weeks gestation (GA)**	**Linear regression effect estimate and CI**							
		Unadjusted				**Adjusted**			
		Estimate	**95% CI**		***p*-value**	**Estimate**	**95% CI**		***p*-value**
Vitamins									
Vitamin B6, mg									
	< 13	0.09	−0.21	0.39	0.5626	0.09	−0.24	0.41	0.6010
	24–28	0.91	0.55	1.27	< 0.0001*	0.82	0.46	1.19	< 0.0001*
	32–36	0.76	0.40	1.11	< 0.0001*	0.58	0.24	0.92	0.0010*
Vitamin C, mg									
	< 13	−0.04	−0.18	0.10	0.5663	−0.08	−0.22	0.07	0.2944
	24–28	0.12	−0.01	0.26	0.0769	0.14	0.02	0.26	0.0252†
	32–36	−0.08	−0.22	0.05	0.2206	−0.08	−0.21	0.05	0.2388

Table 2. continued

Prenatal Serum Markers	Weeks gestation (GA)	Linear regression effect estimate and CI							
		Unadjusted				Adjusted			
		Estimate	95% CI		p-value	Estimate	95% CI		p-value
Vitamin E, αTU									
	< 13	−0.03	−0.12	0.06	0.4596	−0.04	−0.14	0.05	0.3941
	24–28	0.00	−0.10	0.11	0.9521	0.03	−0.07	0.12	0.5710
	32–36	0.05	−0.07	0.16	0.4042	0.05	−0.05	0.16	0.3149
RBC folate, ng/mL									
	< 13	0.00	−0.16	0.17	0.9771	−0.08	−0.25	0.08	0.3209
	24–28	0.07	−0.12	0.25	0.4807	0.12	−0.02	0.26	0.0962
	32–36	−0.06	−0.27	0.16	0.6089	0.01	−0.17	0.18	0.9506
Cytokines and other inflammatory markers									
IL-6, pg/mL									
	< 13	−0.18	−0.42	0.07	0.1582	−0.21	−0.47	0.06	0.1227
	24–28	−0.08	−0.32	0.16	0.5047	0.09	−0.08	0.26	0.2916
	32–36	−0.13	−0.57	0.31	0.5562	0.10	−0.36	0.56	0.6680
IL-8, pg/mL									
	< 13	−0.12	−0.32	0.09	0.2586	−0.12	−0.33	0.10	0.2857
	24–28	−0.01	−0.22	0.20	0.8885	0.04	−0.16	0.25	0.6896
	32–36	0.01	−0.21	0.22	0.9499	0.03	−0.17	0.24	0.7383
CRP, mg/L									
	< 13	−0.29	−0.63	0.04	0.0836	−0.33	−0.67	0.01	0.0548
	24–28	−0.24	−0.57	0.08	0.1367	−0.01	−0.25	0.23	0.9377
	32–36	0.00	−0.32	0.31	0.9885	0.25	0.01	0.49	0.0419
Oxidative stress markers									
PGF2α, ng/mg									
	< 13	−0.19	−0.54	0.15	0.2715	−0.20	−0.57	0.17	0.2810
	24–28	0.03	−0.25	0.31	0.8354	−0.04	−0.36	0.28	0.8171
	32–36	0.02	−0.20	0.24	0.8373	0.09	−0.15	0.33	0.4435
MDA, uM									
	< 13	0.07	−0.09	0.23	0.4016	0.05	−0.12	0.21	0.5883
	24–28	0.00	−0.16	0.17	0.9906	−0.06	−0.21	0.09	0.4087
	32–36	0.04	−0.13	0.22	0.6098	−0.03	−0.20	0.14	0.6989

by randomization group at either time point, with or without adjustment (Table 2a). Per protocol findings were consistent with ITT analysis (Table 2b).

Changes over time. As shown in Fig. 2, these differences were driven by the intervention arm maintaining concentrations of Vitamin B_6 through pregnancy (baseline to 24–28 weeks $p = 0.15$ and baseline to 32–36 weeks $p = 0.19$) compared to the control arm that had a decrease in vitamin B6 concentrations (baseline to 24–28 weeks and baseline to 32–36 weeks $p < 0.0001$). Similarly, Vitamin C concentrations decreased between baseline and 24–28 weeks in the control arm ($p < 0.0001$) but not in the intervention arm ($p = 0.8$).

Cumulative exposure. The intervention led to higher cumulative vitamin B_6 concentrations throughout pregnancy in unadjusted and adjusted analyses compared to control ($p = 0.0005$). Cumulative concentrations of vitamins C, E, and RBC folate did not differ by treatment group.

Secondary outcomes

By-group comparison at each time point. At baseline, the intervention group had lower PGF 2α concentrations (adj β −0.14, 95% CI −0.51, 0.22). The intervention did not impact biomarkers of inflammation (CRP, IL-6, IL-8, or IL-1β) or oxidative stress (PGF 2α or MDA) at either 24–28 weeks or 32–36 weeks (Table 2a, b).

Cumulative exposure. AUC of markers of inflammation or oxidative stress did not differ by randomization group.

Clinical outcomes

In ITT and per-protocol analyses, the intervention did not affect gestational weight gain, rates of gestational diabetes, pregnancy induced hypertension, preterm labor, cesarean section delivery, and exclusive breastfeeding at hospital discharge (Table 3a, b), although our study was not powered for these analyses. Similarly, there was no impact of randomization group on preterm birth rate, gestational age at birth, fetal growth, or neonatal adiposity or lean mass.

Exploratory analyses

In exploratory observational analyses, total AUC vitamin B6 concentrations in the highest vs. lowest quartile was associated with lower birthweight (adj β −0.25, 95% CI −0.47, −0.02) and birthweight z-score (adj β −0.44, 95% CI −0.83, −0.04). Total AUC

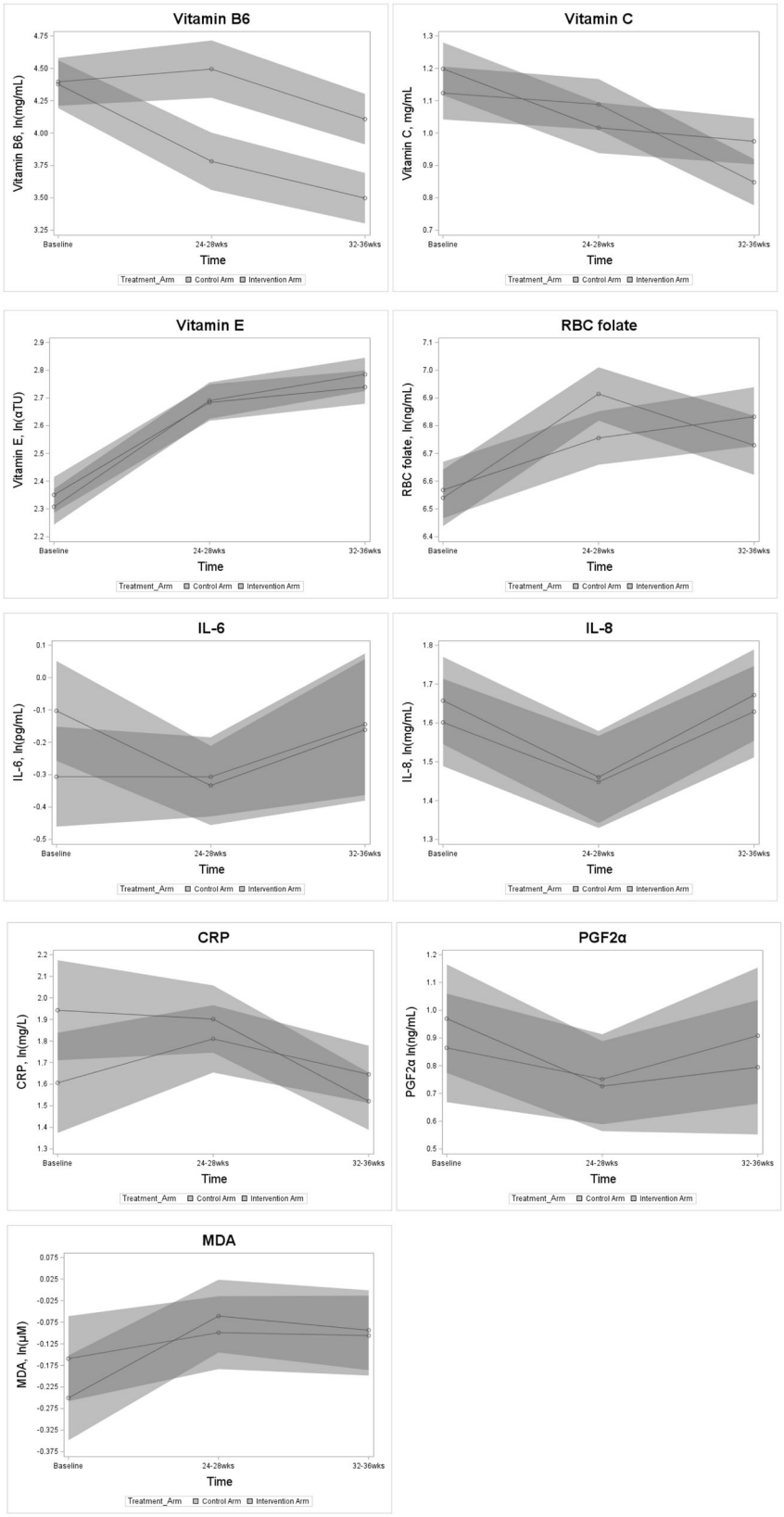

Fig. 2 Pregnancy serum markers (natural log(ln) transformed) by study (PNV) and standard vitamin study arms. Least mean squares and 95% confidence intervals from analysis of multiply imputed data using linear regression models [1].

erythrocyte folate in the highest vs. lowest quartile was associated with higher neonatal length (adj β 0.97, 95% CI 0.42, 1.5) and lower BMI-z (adj β −0.51, 95% CI −1.0, −0.01). Total AUC CRP concentrations in the highest vs. lowest quartile was associated with higher BMI-z (adj β 0.68, 95% CI 0.15, 1.2). Exposure to the highest vs. lowest AUC quartile of IL-8 was associated with higher neonatal fat-free mass (adj β 0.65, 95% CI 0.07, 1.24) and higher subscapular skin fold thickness (adj β 0.86, 95% CI 0.1, 1.6). Exposure to the highest vs. lowest AUC quartile of PGF 2α was associated with lower neonatal fat-free mass (adj β −0.67, 95% CI −1.3, −0.07).

DISCUSSION

Here we report results of an RCT of antioxidant micronutrients C, E, B_6, and folate in pregnant women with obesity with the primary outcome of systemic micronutrient concentrations. The intervention group had higher systemic concentrations of vitamin B_6 throughout pregnancy. In this population, there was no impact of this intervention on markers of inflammation and oxidative stress or on measured clinical outcomes. Exploratory analyses suggested that maternal cumulative inflammatory/oxidative balance over the course of pregnancy may be associated with fetal growth patterns.

Obesity during pregnancy is associated with dysregulated inflammation and oxidative stress, which have been implicated in short- and long-term adverse health outcomes. Lower concentrations of antioxidant micronutrients in individuals with obesity may be related to lower dietary intake of these micronutrients, lower adherence to prenatal vitamin intake [31], differences in volume and compartment of distribution, or differences in inflammation-related metabolism. Human, animal, and tissue models have all suggested that inflammation and oxidative stress may directly decrease concentrations of antioxidant micronutrients [32].

We sought to determine whether providing higher concentrations of specific antioxidant micronutrients to women with obesity increases systemic concentrations of these micronutrients and, secondarily, whether this attenuates inflammation and oxidative stress. We found that the BMI-PNV consistently raised B_6 concentrations only. This may be because the doses of the folate and vitamins C and E that we provided were lower than what was needed to raise systemic concentrations in the setting of obesity. Available data on pharmacokinetics and dynamics in pregnancies complicated by obesity is scant. A recent study reported that the distribution of folate is significantly affected by obesity [33], with a maximum concentration of folate 34% lower in pregnant women with obesity compared to lean women. A study comparing vitamin E pharmacokinetics in lean individuals compared to individuals with obesity was recently completed, revealing that obesity-associated hepatosteatosis leads to sequestration of vitamin E in the liver, although no pregnant participants were included [34]. Given the burden of obesity in the U.S. reproductive-age population and the intergenerational implications of nutritional optimization, our findings highlight the need for more investigation to inform modeling techniques and eventually, supplementation practices [35–38], for this high-risk population.

We additionally did not find that supplementation altered markers of inflammation or oxidative stress. This may be related to the finding that only vitamin B_6 was consistently higher in the intervention group. Vitamins C [39], E [40], B_6, and folate [41] have been found in vitro and in small trials to increase antioxidant capacity, decrease oxidative stress, and attenuate inflammation. We have previously reported in a rodent model that supplementation of obese dams with antioxidant vitamins during pregnancy decreased inflammation and oxidative stress and improved offspring outcomes, including adiposity [13]. Similarly, trials of other antioxidant supplements (coenzyme Q10 [42], melatonin [43], pyrroloquinoline quinone [44]) in mice have reported a decrease in oxidative stress, improved mitochondrial dysfunction in oocytes, and improved reproductive outcomes.

Similar to our findings, combinations of micronutrients in large trials or meta-analyses have not yielded consistent benefit in either inflammatory markers or clinical outcomes [45, 46]. In pregnancy, large trials of vitamin C and E supplementation to prevent preeclampsia have not shown benefit, with one trial showing higher rates of small for gestational age with supplementation [47–50]. Recently, a trial of co-supplementation of magnesium, zinc, calcium, and vitamin D in women with gestational diabetes reported statistically significant decreases in CRP (intervention −1.2 ± 3.5 vs. + control 0.8 ± 2.0 mg/L) and MDA (intervention −0.3 ± 0.3 vs. control 0.3 ± 1.1 μmol/L), although the clinical significance of these differences is unclear [51]. A trial of co-supplementation with vitamin E and 1000 mg omega-3 fatty acids for six weeks in pregnant women with gestational diabetes reported increased total antioxidant capacity and nitric oxide, and decreased MDA [52]. We found only one trial ($n = 51$) targeted women with overweight and obesity and supplemented docosahexanoic and eicosapentanoic acid, with a resultant decrease in markers of inflammation in the intervention arm, suggesting a role for nutrient supplementation beyond micronutrients [53]. Our exploratory findings that cumulative nutrient and biomarker concentrations are associated with modulation of fetal growth suggest that in a population of women with obesity, higher intake of specific nutrients that modulate inflammation and oxidative stress may impact fetal health and development, supporting what has been observed in animal and pre-clinical models [54–58], although more investigation is required as to effective prenatal nutritional interventions targeting inflammation.

The role of dietary supplementation is to replace nutrients that are not consumed in adequate amounts from the diet or to target particular pathologic or biologic pathways to improve health [59]. In pregnancies complicated by obesity, supplementation has the potential to address both of these needs. Higher maternal BMI is associated with lower intake of grains, vegetables, iron, and folate and poor dietary quality, which may worsen in pregnancy, particularly in those of low socioeconomic status [60, 61]. Thus, although pregnant women with obesity would benefit from a healthy and varied diet, many cultural, social and economic barriers may contribute to poorer diet quality. Current nutritional supplement recommendations for women planning pregnancy or early in pregnancy with obesity do not differ from lean women and include a prenatal vitamin with folate and iron [62]. Future studies should embark on both improving access and availability of a healthy, balanced diet and investigation of alternative and adjunct approaches, such as dietary supplementation, to target the unique metabolism and physiology of pregnant women with obesity.

Taken together, available data suggest a role for additional nutrients beyond what we provided in our supplement to abrogate inflammation in pregnancy. More well-powered studies in women with obesity are urgently needed to determine effective combinations of supplementation to decrease inflammation and delineate the effect on maternal and infant health outcomes.

Strengths of our study include the double-blind, placebo controlled RCT design, recruitment in early pregnancy with a relatively long exposure to the intervention, longitudinal biomarker and systemic micronutrient measurements with rigorous laboratory methodology, and thorough maternal and infant outcome ascertainment. Our subjects were sociodemographically representative of populations with obesity, supporting the generalizability of our findings. Our study was limited by imbalanced baseline infections between randomization groups, for which we attempted to statistically adjust, but may have biased our findings towards the null. We were also limited by

Table 3. Effect of intervention on maternal and child outcomes.

	Total Sample $n = 98$				Treatment Arm: Study Vitamin (PNV) $n = 52$				Treatment Arm: Standard Vitamin (reference) $n = 46$				Unadjusted			Adjusted[2]		
	n	Median	Interquartile range		*n*	Median	Interquartile range		*n*	Median	Interquartile range		Estimate	95% CI		Estimate	95% CI	
Pregnancy Outcomes																		
Change in weight, kg (Baseline-Delivery)	**95**	**9.3**	**5.5**	**14.2**	**49**	**9.5**	**5.1**	**15.4**	**46**	**9.2**	**5.9**	**14.0**	**0.2**	**−2.8**	**3.2**	**−0.4**	**3.4**	**2.6**
	Total Sample $n = 102$				**Treatment Arm: Study Vitamin (PNV) $n = 52$**				**Treatment Arm: Standard Vitamin (reference) $n = 50$**				**Unadjusted**			**Adjusted[1]**		
	n	Median	Interquartile range		*n*	Median	Interquartile range		*n*	Median	Interquartile range		Estimate	95% CI		Estimate	95% CI	
Infant Outcomes[1,3.]																		
Gestational age, weeks	102	38.4	37.1	39.6	52	38.6	37.2	39.5	50	38.4	37.1	39.6	0.3	−0.7	1.3	0.1	−0.8	1.1
Birthweight, kg	101	3.3	2.9	3.7	51	3.3	2.8	3.7	50	3.3	3.0	3.6	0.0	−0.3	0.3	0.1	−0.3	0.4
Weight z score	100	0.1	−0.4	0.7	50	0.2	−0.5	0.7	50	0.1	−0.3	0.6	0.1	−0.3	0.6	0.0	−0.5	0.5
Length z score	97	−0.1	−0.7	0.7	50	−0.1	−0.5	0.6	47	−0.1	−0.8	0.7	0.0	−0.6	0.6	0.0	−0.7	0.6
BMI z score	97	0.3	−0.2	1.0	50	0.3	-0.4	1.0	47	0.3	-0.2	1.0	0.1	−0.5	0.6	0.1	−0.6	0.7
Fat mass	56	0.3	0.2	0.5	26	0.4	0.2	0.5	30	0.3	0.2	0.5	0.0	−0.1	0.2	0.0	−0.1	0.2
Fatfree Mass	56	2.8	2.6	3.2	26	2.7	2.5	3.0	30	2.9	2.6	3.2	−0.2	−0.5	0.2	−0.2	−0.6	0.2
Bicep, average	78	8.9	8.0	10.0	37	9.0	8.0	10.2	41	8.8	8.0	9.8	0.2	−0.6	0.9	−0.2	−0.9	0.6
Tricep, average	77	9.7	9.0	10.5	36	9.7	9.2	10.6	41	9.8	8.8	10.2	−0.2	−1.0	0.6	0.3	−0.6	1.3
Iliac, average	78	8.0	7.7	9.0	37	8.0	7.3	9.0	41	8.0	7.8	8.7	0.0	−0.6	0.6	0.0	−0.7	0.7
Subscapular, average	77	9.5	8.8	10.5	36	9.3	8.5	10.2	41	9.8	9.2	10.5	−0.5	−1.2	0.2	−0.3	−1.1	0.5
Apgar score, 1 min	101	8.0	7.0	8.0	51	8.0	7.0	8.0	50	8.0	7.0	9.0	na			na		
Apgar score, 5 min	101	9.0	9.0	9.0	51	9.0	8.0	9.0	50	9.0	9.0	9.0	na			na		

	Total Sample $n = 98$		**Treatment Arm: Study Vitamin (PNV) $n = 52$**		**Treatment Arm: Standard Vitamin (reference) $n = 46$**		**Unadjusted**			**Adjusted[3.]**		
	n	%	*n*	%	*n*	%	OR	95% CI		OR	95% CI	
Pregnancy Outcomes												
Hypertension during pregnancy	18	18.4%	13	25.0%	5	10.9%	2.7	0.9	8.4†	2.7	0.8	8.9†
HELLP	0	0.0%	0	0.0%	0	0.0%						
no proteinuria	12	12.2%	8	15.4%	4	8.7%	1.9	0.5	9.3	1.5	0.3	7.7
with proteinuria	6	6.1%	5	9.6%	1	2.2%	4.7	0.5	231.4	8.6	0.7	539.4
Gestational diabetes	17	17.7%	9	17.3%	8	17.4%	1.0	0.3	2.8	1.3	0.4	4.0
Mode of delivery, Caesarean section v. vaginal (ref)	37	37.8%	21	40.4%	16	34.8%	1.3	0.6	2.9	1.6	0.6	3.8
Infection, post-partum	1	1.1%	1	2.1%	0	0.0%	na			na		
Thromboses, post-partum	0	0.1%	0	0.0%	0	0.0%	na			na		

Table 3. continued

	Total Sample *n* = 98		**Treatment Arm: Study Vitamin (PNV) *n* = 52**		**Treatment Arm: Standard Vitamin (reference) *n* = 46**		**Unadjusted**			**Adjusted[3.]**		
	n	**%**	***n***	**%**	***n***	**%**	**OR**	**95% CI**		**OR**	**95% CI**	
	Total Sample *n* = 102		Treatment Arm: Study Vitamin (PNV) *n* = 52		Treatment Arm: Standard Vitamin (reference) *n* = 50		Unadjusted			Adjusted[3.]		
	n	%	*n*	%	*n*	%	OR	95% CI		OR	95% CI	
Infant Outcome[1., 2.]												
Preterm, < 37 weeks GA	17	16.7%	9	17.3%	8	16.0%	1.4	0.5	4.3	na		
Centile												
SGA	0	0.0%	0	0.0%	0	0.0%	na			na		
LGA	15	15.0%	9	18.0%	6	12.0%	1.5	0.5	4.7	2.3	0.6	8.7

*$p < 0.05$. (Table 3a)

[1]Median regression analyses performed using STATA qreg wrapper qreg2 for clustered standard errors (Stata v17.0, StatCorp, College Station, TX). (Table 3a)

[2]Adjusted analyses controlled for maternal educational attainment (college degree or higher, Yes/No), and baseline infections, prenatal vitamins, and analgesic medication. Analyses of infant outcomes are adjusted for intrafamilial correlation among twins. (Table 3a)

[3]Z scores based on Olsen reference. (Table 3a)

*$p < 0.05$, $^{\dagger}p < 0.10$. (Table 3b)

[4]Model run using logistic regression; exact logistic regression used for analyses where cell size < 5. Analyses of infant outcomes were run using generalized estimating equations with an exchangeable covariance structure to account for intrafamilial correlation among multiple births. (Table 3b)

[5]Adjusted analyses controlled for maternal educational attainment (college degree or higher, Yes/No), and baseline infections, prenatal vitamins, and analgesic medication. Analyses of infant outcomes are adjusted for intrafamilial correlation among twins. (Table 3b)

[6]Z scores based on Olsen reference. (Table 3b)

[a]Effect of intervention on continuous pregnancy and infant outcomes in $n = 98$ mothers who completed the trial and their $n = 102$ infants

[b]Effect of intervention on categorical pregnancy and infant outcomes among $n = 98$ women who completed study and their $n = 102$ infants.

loss to follow-up, although the characteristics of those who did not complete the study did not differ from those who did, and participants were blinded to outcome status, suggesting that this was not a source of bias. Although we attempted to collect dietary intake data, the high rate of missing data for this questionnaire limited inference.

Here we report that co-supplementation with vitamins B_6, C, E and folate in pregnancies complicated by obesity raised systemic concentrations of vitamin B_6 and C but did not affect inflammation or oxidative stress markers or clinical outcomes. Our findings suggest that more foundational knowledge is needed regarding the biochemical impact of nutritional interventions in pregnancies complicated by obesity to inform dosing strategies. Further, our findings highlight the need to partner with pregnant women with obesity to innovate approaches to target their unique metabolic needs and improve health outcomes for two generations.

DATA AVAILABILITY

Limited data sets and data dictionaries will be made available by secure, electronic transmission from ten years of study publication to investigators who provide a detailed analysis plan that is agreed upon by the co-authors of this manuscript.

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ACKNOWLEDGEMENTS

We would like to thank the participants, without whom this study would not have been possible. We would also like to thank Annie Penfield-Cyr, MS and Charis Benjamin, BS for their important contributions to this study.

AUTHOR CONTRIBUTIONS

SS, SC, MH, MRH, TM, RF, EO, and SNM have all contributed to the study design, data acquisition, interpretation of results, and writing or revising the manuscript.

FUNDING

SS was supported by K23 HD 074648, K23 HD 074648 S1, and The Charles Hood Foundation.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41366-024-01472-z.

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