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## Vitamin B<sub>12</sub> Status and Metabolic Health in Women of Reproductive Age: Population-Based Biomarker Survey

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### Abstract

**Background and aims:** In clinical populations, vitamin B<sub>12</sub> deficiency has been associated with adverse metabolic health (e.g., gestational diabetes). Population-level data among women of reproductive age could inform screening and interventions. The objective of this analysis was to examine the prevalence of adverse metabolic characteristics (elevated adiposity and central adiposity, hypertension, elevated glycated hemoglobin [HbA1c]) and associations of vitamin B<sub>12</sub> status with metabolic characteristics in women as part of a population-based biomarker survey in Southern India.

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**Methods:** Participants (n=980 women 15–40y; not pregnant or lactating) were assessed for total vitamin B<sub>12</sub>, holotranscobalamin, methylmalonic acid, homocysteine, and HbA1c. Categorical anthropometry assessments and bioelectrical impedance analysis (e.g., whole body (WF%) and trunk (TF%) fat) were assessed among adults (≥18y). Linear and binomial regressions were used to examine associations of vitamin B<sub>12</sub> status with metabolic characteristics.

**Results:** Overall, 25% of participants had HbA1c ≥5.7% (HbA1c≥5.7-<6.5%: 20.0%; ≥6.5%: 5.0%), and 18.6% had hypertension (Stage 1: 16.4%; Stage 2: 2.2%). Among adults, 23.4% had body mass index of (BMI) 25.0–<30.0 kg/m<sup>2</sup>, 9.6% had BMI ≥30.0 kg/m<sup>2</sup>, 13.4% had elevated waist circumference (WC; >88.9cm), and 20.8% had elevated waist-hip ratio (WHR; ≥0.85cm). Overall, higher vitamin B<sub>12</sub> concentrations were associated with lower BMI and WC. Among adults, higher vitamin B<sub>12</sub> concentrations were associated with lower WF% and TF%; and lower prevalence of overweight (BMI≥25.0 kg/m<sup>2</sup>) and elevated WC, WHR, and WF%. Similarly, vitamin B<sub>12</sub> <148 pmol/L was associated with higher BMI and WC overall and, among adults, higher WF% and TF%, and increased overweight (BMI≥25.0 kg/m<sup>2</sup>; prevalence ratio: 1.31; 95% confidence interval: 1.09–1.58), and elevated WC (>88.9 cm; 1.85[1.32–2.60]), WHR (≥85.0; 1.38[1.07–1.78]), WF% (>35%; 1.29[1.10–1.51]), and TF% (>35%; 1.25[1.06–1.49]).

**Conclusions:** The burden of adverse metabolic characteristics was substantial in this population of young, apparently healthy women. Among those with vitamin B<sub>12</sub> <148 pmol/L there was increased central adiposity and overweight status. Evaluating vitamin B<sub>12</sub> and metabolic outcomes prospectively could inform screening and interventions to improve women's health.

**Registration number:** [NCT04048330](#)

## Keywords

Vitamin B<sub>12</sub>; body composition; metabolic health; overweight; periconceptional

## INTRODUCTION

Vitamin B<sub>12</sub> deficiency (<148 pmol/L) is an important public health problem worldwide (1–3) with the highest burden in pregnant women, young children, and the elderly (1–3). Vitamin B<sub>12</sub> deficiency classically presents as pernicious or megaloblastic anemia (4), and has been linked to neurological impairments and risk of adverse pregnancy outcomes, including neural tube defects (3–7).

Vitamin B<sub>12</sub> is a required cofactor for methionine synthase and L-methyl-malonyl-coenzyme A mutase (3), and is required for nucleotide biosynthesis, methyl donor production, folate-mediated one-carbon metabolism, homocysteine flux, and fatty acid and amino acid metabolism (3, 8, 9). Vitamin B<sub>12</sub> may have an important role in other aspects of metabolism, including gut microbiome (10) and metabolic health (11, 12). Emerging evidence from murine (13–18) and cellular (i.e., adipocytes, hepatocytes) models (19–21) has identified a potential role of vitamin B<sub>12</sub> status in insulin resistance, dyslipidemia, and adipocyte dysfunction, although the mechanisms have yet to be established.

Evidence from studies in clinical (i.e., patients with metabolic comorbidities) and obstetric populations suggests that lower vitamin B<sub>12</sub> status may be associated with adverse metabolic

health outcomes. In adults with obesity or metabolic comorbidities, lower vitamin B<sub>12</sub> status has been associated with higher insulin resistance (22–25), glycated hemoglobin (HbA1c) (22), fasting glucose (22, 26), and waist circumference (26). During pregnancy, vitamin B<sub>12</sub> deficiency has been associated with gestational diabetes and insulin resistance (27–34). In small clinical trials, vitamin B<sub>12</sub> supplementation improved insulin resistance and HbA1c compared to placebo in adults with type 2 diabetes (n=57, 8 weeks) (35), and improved fasting glucose (but not insulin resistance) in adults with non-alcoholic fatty liver disease, compared to baseline but not to placebo (n=40, 12 weeks) (36).

There are limited population-level data on the role of vitamin B<sub>12</sub> in metabolic health, including in women of reproductive age (37–43). Research to date has been constrained by limited assessment of indicators of vitamin B<sub>12</sub> status and metabolic health. To date, two population-based studies have been conducted in women of reproductive age (15–49 y; Vietnam) (40) or premenopausal women (≥19 y; USA) (43), which assessed body mass index (BMI). Although BMI is used as a common screening indicator for obesity-related health risks, it does not differentiate between body lean and fat mass, and indicators of central adiposity (e.g., waist circumference) have been linked to metabolic health outcomes, independent of BMI (44–47). There are also limited data from settings such as India, where the burden of vitamin B<sub>12</sub> deficiency and diabetes are estimated to be among the highest globally (48–52). Evaluating the role of vitamin B<sub>12</sub> in the development of metabolic health outcomes could inform screening and interventions, particularly in women of reproductive age, given the importance of preconception nutrition for longer-term health outcomes in women and their children.

The objectives of this analysis were to examine metabolic characteristics (adiposity, central adiposity, blood pressure, glycated hemoglobin), and to evaluate the associations of vitamin B<sub>12</sub> status with metabolic characteristics in women, as part of a population-based biomarker survey in Southern India.

## METHODS

### Study design and population.

This analysis was conducted within a cross-sectional population-based biomarker survey of non-pregnant, non-lactating women 15–40 years of age, in Andhra Pradesh, India ([NCT04048330](#)). The study design and findings have been previously described (48, 53, 54). Briefly, trained nurse enumerators conducted a census among all households (rural: 3,124; urban: 3,428) within the catchment area of the research site, Arogyavaram Medical Centre (AMC). All rural households (n=3,124) and a simple random sample of urban households (n=1,000) were revisited for screening and recruitment. One woman (15–40 y, not pregnant, not lactating) per household was selected using an *a priori* algorithm developed by a biostatistician at the U.S. Centers for Disease Control and Prevention (CDC; CER). Study staff referred women who were currently pregnant or had severe anemia (Hb<8.0 g/dL) to a local clinic for standard of care.

## Ethics.

The study protocol ([NCT04048330](#)) was approved by the Institutional Review Board at Cornell University and the Institutional Ethics Committees at St. John's Research Institute and AMC; and received clearance from the Indian Council of Medical Research Health Ministry's Screening Committee. The protocol was reviewed in accordance with the U.S. Centers for Disease Control and Prevention (CDC) human research protection procedures and was determined to be a non-research, routine surveillance activity. A non-disclosure agreement for personally identifiable information and a data sharing agreement for de-identified data were established. All participants provided written informed consent or assent prior to data collection.

## Data collection.

Data were collected at a single study visit at AMC by trained nurse enumerators on electronic tablets (55), and included sociodemographic, clinical, anthropometric, and dietary data. Anthropometric measurements were collected in triplicate and included weight using a calibrated digital scale to the nearest 0.01 kg (Seca 874DR, Hamburg, Germany), height using a stadiometer to the nearest 0.1 cm (Weigh and Measure LLC, Olney, MD); waist and hip circumferences using a retractable, non-stretch measuring tape to the nearest 0.1 cm; mid-upper arm circumference using a 65 cm measuring tape to the nearest 0.1 cm (Weigh and Measure LLC, Olney, MD); and triceps skinfold using a caliper to the nearest 0.5 mm (Lange Skinfold Caliper; Beta Technology, Santa Cruz, CA). Bioelectrical impedance analysis (BIA) was used to evaluate whole body (WF%) and trunk (TF%) percent fat mass in women  $\geq 18$  y (BC-418 MA; Tanita Corporation, Tokyo, Japan; 8 electrode). Diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured in triplicate using a manual sphygmomanometer.

## Laboratory analyses.

Venous blood was collected, processed, and stored using standardized protocols (53). Hemoglobin (Hb) and glycated hemoglobin (HbA1c) were evaluated in real-time *via* Coulter Counter (Coulter HMX) and nephelometry (Agappe Diagnostics, Kerala, India), respectively. Vitamin B<sub>12</sub> biomarkers were analyzed in archived samples ( $\leq -80^{\circ}\text{C}$ ) in batch after the completion of data collection, including serum total vitamin B<sub>12</sub> *via* chemiluminescence (E411Roche Diagnostics, Mannheim, Germany), plasma methylmalonic acid (MMA) and homocysteine (Hcy) *via* gas chromatography-mass spectrometry (GCMS-SQ, 5975, Agilent Technologies, CA USA), and plasma holotranscobalamin (holoTC) *via* an enzyme-immunoassay (ELISA, Abbexa). Red blood cell (RBC) folate and serum folate concentrations were measured *via* the World Health Organization (WHO)-recommended microbiologic assay (56). Ranges and mean coefficients of variation for quality controls for each assay were: vitamin B<sub>12</sub> (pmol/L; low: 218–328, 7.5%, medium: 238–439, 6.1%, high: 448–615, 4.5%), MMA ( $\mu\text{mol/L}$ ; pooled: 0.41–0.52, 3.7%), Hcy ( $\mu\text{mol/L}$ ; pooled: 6.82–8.10, 2.8%), RBC folate (nmol/L; low: 127–300, 8.8%; high: 322–595, 7.5%), and serum folate (nmol/L; low: 5.6–11.3, 7.4%; high: 20.6–41.9, 7.1%).

### Definitions of exposures.

Vitamin B<sub>12</sub> was categorized as <148 and <221 pmol/L (57, 58). Elevated MMA, a functional biomarker of vitamin B<sub>12</sub> status, was defined as >0.26 µmol/L (3, 58). Both circulating and functional biomarkers are recommended for assessing vitamin B<sub>12</sub> status (3, 57, 58); impaired vitamin B<sub>12</sub> status was defined as vitamin B<sub>12</sub> <148 pmol/L and MMA >0.26 µmol/L. Other cutoffs for lower vitamin B<sub>12</sub> status were also evaluated (MMA >0.26 and >0.37 µmol/L (3, 58); holotTC <35 and <40 pmol/L (3, 58); combined vitamin B<sub>12</sub> indicator, cB<sub>12</sub> ≤ -1.5 (59); vitamin B<sub>12</sub> ≥148-<221 pmol/L); results were not significant and were not reported. Vitamin B<sub>12</sub> status as a three-level variable (<148, ≥148-<221, and ≥221 pmol/L) and quintiles were also considered; results were similar to primary analyses and were not reported. Elevated Hcy was defined as >10.0 and >15.0 µmol/L (60). RBC folate was categorized as <305 and <748 nmol/L, respectively, and serum folate was categorized as <7 nmol/L (60, 61).

### Definitions of metabolic characteristics.

Continuous measures of body mass index (BMI; weight [kg] / height [m]<sup>2</sup>), waist circumference (WC), and waist-hip ratio (WHR) were examined among all women (≥15 y). Categorical measures of BMI, WC, and WHR, which were established for adults, were examined among adult women (≥18 y). Among adult women (≥18 y), BMI was categorized using WHO criteria (i.e., BMI <18.5, 18.5 to <25.0, 25.0 to <30.0, and ≥30.0 kg/m<sup>2</sup>) (62) for comparability across studies; analyses using BMI cut-offs for Asian populations (63) had similar findings (data not shown). Elevated waist circumference (WC) was defined as >88.9 cm (converted from >35 inches) (64) and elevated WHR was defined as ≥0.85 (65), based on NIH and WHO criteria.

Continuous and categorical measures of WF% and TF%, which were assessed using a BIA model calibrated for ages 18–84 y, were examined among adult women (≥18 y). Elevated WF% and TF% were defined among adult women (≥18 y) as >35%, as suggested by the WHO and American Association of Clinical Endocrinology (66–69).

Among all women (≥15 y), blood pressure was categorized as normal (SBP <120 and DBP <80 mmHg), elevated blood pressure (SBP 120–129 and DBP <80 mmHg), stage 1 hypertension (SBP 130–139 or DBP 80–89 mmHg), and stage 2 hypertension (SBP ≥140 or DBP ≥90 mmHg) (70). Hypertension defined as SBP ≥130 or DBP ≥80 mm Hg (i.e., stage 1 or 2 hypertension) was also considered in analyses. Among all women, HbA1c was categorized as <5.7%, ≥5.7% to <6.5%, and ≥6.5%, using WHO and American Diabetes Association criteria corresponding to cutoffs for normoglycemia, prediabetes, and diabetes, respectively (71, 72); HbA1c ≥5.7% was also considered in analyses.

### Statistical analyses.

Continuous variables were categorized using conventional cutoffs, where available. Biomarker values that were below or above the limits of detection (LOD) for assays were set to half the LOD or 2-times the LOD, respectively, and continuous biomarker values were natural logarithmically transformed prior to analyses. Other variables were assessed for skewness and determined to be normally distributed and were not transformed. Continuous

variables were presented as geometric means (GM) and 95% confidence intervals (95% CI). Chi-square statistics and one-way ANOVA were used to evaluate differences in categorical and continuous metabolic characteristics, respectively, by rural/urban residence and vitamin B<sub>12</sub> status.

Linear and binomial regression models were used to examine the associations of vitamin B<sub>12</sub> status (continuous and categorical) with continuous and categorical metabolic characteristics (adiposity, central adiposity, blood pressure, glycated hemoglobin), respectively. Results from models are presented as  $\beta$  estimates and standard error (unit change in continuous metabolic characteristics) and prevalence ratios and 95% confidence intervals (categorical metabolic characteristics). Variables were evaluated as potential effect measure modifiers (e.g., rural/urban residence, parity, BMI  $\geq 25.0$  kg/m<sup>2</sup> categories, folate status); effect modification was not detected (p-value for interaction term  $< 0.10$ ). Potential confounders were evaluated using the approach recommended by Rothman and Greenland (73), in which risk factors for an outcome (i.e., factors associated with an outcome in prior literature) that led to a  $> 10\%$  change in estimate were included in models. The following variables were considered for inclusion in multivariable models: age, education, currently married, gravidity, employment status. Results were similar when considering rural/urban residence and dietary variables (vegetarian dietary preference and frequency of animal-source food consumption) for inclusion in multivariable models; therefore, parsimonious models without these variables are presented. Potential nonlinear associations of vitamin B<sub>12</sub> status with metabolic characteristics were investigated using restricted cubic splines and assessed using the likelihood ratio test, comparing models with only the linear term to models with both the linear and cubic spline terms (74). Observations with missing data for covariates ( $< 3\%$  per variable, i.e., education,  $n=5$ ; marital status,  $n=5$ ; gravidity,  $n=5$ ; employment status,  $n=28$ ) were retained in analyses using the missing indicator method (i.e., in which a dummy variable to indicate missing and a variable with missing values set to the reference value were included in the model) (75). The findings for the complete case analysis were similar to those from the analysis using the missing indicator method; the results based on the missing indicator method (i.e., retaining missing data in the analysis) are reported. Sensitivity analyses were conducted among adults ( $\geq 18$  y), and among women with BMI  $\geq 18.5$  kg/m<sup>2</sup>.

Statistical analyses were conducted using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). Analyses were reproduced by the Cornell Center for Social Sciences (CCSS) Data Archive and Replication Team.

## RESULTS

### Participants characteristics.

Characteristics of women in this study are provided in Table 1 and Supplemental Table 1, and a flow chart of study participants is presented in Supplemental Figure 1. Most women had some formal schooling (83.8%), were currently married (79.4%), and had 4 to 5 family members in their household. A total of 93.1% of women reported following a non-vegetarian diet (i.e., consumption of meat, poultry, or fish), although consumption of animal-source foods was low ( $< 25\%$  of women consumed eggs, poultry, meat, or fish  $\geq 1$  time per week). A

total of 48.3% of women had vitamin B<sub>12</sub> concentrations <148 pmol/L, 74.3% had vitamin B<sub>12</sub> concentrations <221 pmol/L; and 37.8% of women had impaired vitamin B<sub>12</sub> status (i.e., vitamin B<sub>12</sub> <148 pmol/L and MMA >0.26 µmol/L).

### Metabolic characteristics.

Metabolic characteristics of the study population are presented in Table 2. A total of 23.4% of adult women (≥18 y) had overweight (BMI ≥25.0 to <30.0 kg/m<sup>2</sup>) and 9.6% had obesity (BMI ≥30.0 kg/m<sup>2</sup>). A total of 13.4% of adult women had elevated WC (>88.9 cm), 20.8% had elevated WHR (≥0.85), 39.3% had elevated WF% (>35%), and 36.2% had elevated TF% (>35%). In the overall population, one-fourth of women had HbA1c ≥5.7% (HbA1c ≥5.7 to <6.5%: 20.0%; HbA1c ≥6.5%: 5.0%); and 18.6% had hypertension (Stage 1: SBP 130–139 or DBP 80–89 mmHg: 16.4%; Stage 2 (SBP ≥140 or DBP ≥90 mmHg: 2.2%). Women living in urban settings had higher BMI and WC compared to women in rural areas (p<0.05), and adult women living in urban settings had higher WF% compared to adult women in rural areas (p<0.05).

### Metabolic characteristics by vitamin B<sub>12</sub> <148 pmol/L.

Metabolic characteristics by vitamin B<sub>12</sub> strata (<148 pmol/L vs. ≥148 pmol/L) are presented in Figures 1 and 2. Women with vitamin B<sub>12</sub> concentrations <148 pmol/L had higher BMI and WC compared to women with vitamin B<sub>12</sub> ≥148 pmol/L (p<0.05). Adult women with vitamin B<sub>12</sub> concentrations <148 pmol/L had higher WF% and TF%, and higher prevalence of overweight or obesity, and elevated WC, WHR, WF%, and TF%, compared to adult women with vitamin B<sub>12</sub> ≥148 pmol/L (p<0.05) (Supplemental Table 2). Other metabolic characteristics, including HbA1c, blood pressure, and hypertension, did not differ by vitamin B<sub>12</sub> <148 pmol/L. Results were similar when examining metabolic characteristics across three levels of vitamin B<sub>12</sub> status (<148, ≥148–<221, and ≥221 pmol/L; Supplemental Table 3).

### Total vitamin B<sub>12</sub> concentrations and metabolic characteristics.

The associations between continuous total vitamin B<sub>12</sub> concentrations and metabolic characteristics are presented in Table 3. Nonlinear associations were not detected; continuous vitamin B<sub>12</sub> concentrations were associated with metabolic characteristics consistently across strata of vitamin B<sub>12</sub> status (Supplemental Figure 2). Higher continuous vitamin B<sub>12</sub> concentrations were associated with lower indicators of central adiposity and overweight including WC (β: −1.64; standard error [SE]: 0.61; p=0.007), in multivariable models adjusting for age, education, currently married, gravidity, and employment, and BMI (β [SE]: −0.92 [0.27]; p=0.001), in multivariable models adjusting for age, currently married, gravidity, and employment; and lower WF% (β [SE]: −1.34 [0.49]; p=0.007) and TF% (β [SE]: −1.51 [0.61]; p=0.013), in multivariable models among adults adjusting for currently married, gravidity, and employment. Among adult women, higher continuous vitamin B<sub>12</sub> concentrations were associated with lower prevalence of overweight (prevalence ratio [PR]: 0.82; 95% confidence interval [95% CI]: 0.71, 0.96; p=0.013), and elevated WC (PR: 0.64 [95% CI: 0.49, 0.85]; p=0.002), WHR (PR: 0.81 [95% CI: 0.66, 1.00]; p=0.049), and WF% (PR: 0.86 [95% CI: 0.76, 0.98]; p=0.028) in multivariable models. Continuous

total vitamin B<sub>12</sub> concentrations were not associated with HbA1c, elevated HbA1c, systolic or diastolic blood pressure, or hypertension.

### **Vitamin B<sub>12</sub> <148 pmol/L and metabolic characteristics.**

The associations between vitamin B<sub>12</sub> <148 pmol/L and metabolic characteristics are presented in Table 4. Women with vitamin B<sub>12</sub> concentrations <148 pmol/L had 1.29 kg/m<sup>2</sup> higher BMI ( $\beta$  [SE]: 1.29 [0.32];  $p<0.0001$ ) in multivariable models adjusting for age, currently married, and employment, and higher WC ( $\beta$  [SE]: 2.65 [0.72];  $p<0.001$ ) in multivariable models adjusting for age, currently married, gravidity, and employment. Adults with vitamin B<sub>12</sub> concentrations <148 pmol/L had higher WF% ( $\beta$  [SE]: 1.72 [0.60];  $p=0.004$ ) and TF% ( $\beta$  [SE]: 1.99 [0.73];  $p=0.007$ ) compared to adult women with vitamin B<sub>12</sub>  $\geq 148$  pmol/L. Similarly, in adult women, vitamin B<sub>12</sub> concentrations <148 pmol/L was associated with increased prevalence of overweight (PR: 1.31 [95% CI: 1.09, 1.58];  $p=0.004$ ), and elevated WC (PR: 1.85 [95% CI: 1.32, 2.60];  $p<0.001$ ), WHR (PR: 1.38 [95% CI: 1.07, 1.78];  $p=0.013$ ), WF% (PR: 1.29 [95% CI: 1.10, 1.51];  $p=0.001$ ), and TF% (PR: 1.25 [95% CI: 1.06, 1.49];  $p=0.010$ ), compared to vitamin B<sub>12</sub>  $\geq 148$  pmol/L in multivariable models. Vitamin B<sub>12</sub> <148 pmol/L was not significantly associated with other metabolic characteristics, including HbA1c, blood pressure, and hypertension.

### **Impaired vitamin B<sub>12</sub> status and metabolic characteristics.**

The associations between impaired vitamin B<sub>12</sub> status (vitamin B<sub>12</sub> <148 pmol/L and MMA >0.26  $\mu$ mol/L) and metabolic characteristics are presented in Table 5. Impaired vitamin B<sub>12</sub> status was associated with higher BMI ( $\beta$  [SE]: 0.73 [0.34];  $p=0.031$ ) and higher WF% ( $\geq 18$  y;  $\beta$  [SE]: 1.28 [0.61];  $p=0.036$ ) and TF% ( $\geq 18$  y;  $\beta$  [SE]: 1.51 [0.76];  $p=0.046$ ) in multivariable models. Adult women with impaired vitamin B<sub>12</sub> status had higher prevalence of overweight (PR: 1.22 [95% CI: 1.02, 1.46];  $p=0.032$ ), elevated WC (PR: 1.48 [95% CI: 1.07, 2.06];  $p=0.020$ ), and elevated TF% (PR: 1.20 [95% CI: 1.02, 1.42];  $p=0.033$ ) in multivariable models. Impaired vitamin B<sub>12</sub> status was not significantly associated with other metabolic characteristics, including HbA1c, blood pressure, or hypertension.

### **Other cutoffs and biomarkers of vitamin B<sub>12</sub> status and metabolic characteristics.**

The associations between vitamin B<sub>12</sub> <221 pmol/L and metabolic characteristics are presented in Supplemental Table 4. Women with vitamin B<sub>12</sub> <221 pmol/L had higher BMI ( $\beta$  [SE]: 1.04 [0.37];  $p=0.006$ ) and WC ( $\beta$  [SE]: 2.09 [0.83];  $p=0.012$ ). Adult women with vitamin B<sub>12</sub> <221 pmol/L had higher WF% ( $\beta$  [SE]: 1.59 [0.67];  $p=0.018$ ), and TF% ( $\beta$  [SE]: 1.88 [0.83];  $p=0.024$ ), and higher prevalence of elevated WC (PR: 1.54 [95% CI: 1.00, 2.36];  $p=0.037$ ), compared to adult women with vitamin B<sub>12</sub>  $\geq 221$  pmol/L in multivariable models. Lower vitamin B<sub>12</sub> status defined using other biomarkers or cutoffs (MMA >0.37  $\mu$ mol/L; holotc <35 and <40 pmol/L; vitamin B<sub>12</sub>  $\geq 148$ -<221 pmol/L; combined vitamin B<sub>12</sub> indicator, cB<sub>12</sub>  $\leq -1.5$ ) were not associated with indicators of adiposity, HbA1c, or blood pressure (data not shown). Vitamin B<sub>12</sub> status as a three-level variable (<148,  $\geq 148$ -<221, and  $\geq 221$  pmol/L) and quintiles were similar to primary analyses (data not shown).

### Sensitivity analyses.

Findings from all sensitivity analyses (among women  $\geq 18$  y, Supplemental Table 5; and among women with BMI  $\geq 18.5$  kg/m<sup>2</sup>, data not shown) were similar to the full population.

## DISCUSSION

In this population-based study of women in Southern India, there was a substantial burden of overweight (33%), elevated central adiposity (21%), and adverse metabolic characteristics (HbA1c  $\geq 5.7\%$ : 25%). Vitamin B<sub>12</sub>  $< 148$  pmol/L (48.3%) was associated with overweight and elevated central adiposity, but not HbA1c or blood pressure. The prevalence of obesity and diabetes continues to increase in low- and middle-income settings (76–78), where the burden of vitamin B<sub>12</sub> deficiency is high (3), availability, access, and uptake to vitamin B<sub>12</sub>-rich foods are limited (58), and coverage of micronutrient fortification programs may be low (79, 80). Understanding the role of vitamin B<sub>12</sub> in metabolic characteristics could help inform programs to improve nutritional and metabolic health.

### Metabolic characteristics.

The prevalence of overweight and obesity (78, 81), diabetes (76, 77, 82), and hypertension (83, 84) have increased globally. In this population, the prevalence of overweight in adult women ( $\geq 18$  y; BMI  $\geq 25$  kg/m<sup>2</sup>: 33.0%) was similar to state-level estimates in women 15–49 y in India (NFHS-5; Andhra Pradesh: 36.3%) (85, 86) and in other population-based studies in Southern India ( $\geq 18$  y: 28.4–42.2%) (87, 88). In contrast, the prevalence of central adiposity among adult women in the current study (WC  $> 88.9$  cm: 13.4%; WHR  $\geq 0.85$ : 20.8%) was lower than state-level estimates (WC  $> 88.0$  cm: 28.4%; WHR  $\geq 0.85$ : 48.8%) (85, 86); findings from other population-based studies have varied (87–92). Similar to the current study, state-level estimates for overweight and elevated waist circumference were higher for women living in urban settings compared to rural settings (86). The prevalence of elevated HbA1c corresponding to cutoffs for prediabetes ( $\geq 5.7\%$ : 25.0%) and diabetes ( $\geq 6.5\%$ : 4.9%) screening in women  $\geq 15$  y in this study was lower than other population-based studies in women in West Bengal ( $\geq 18$  y;  $\geq 5.7\%$ : 34.8%;  $\geq 6.5\%$ : 9.7%) (93) and Mysore (30–59 y;  $\geq 5.7\%$ : 57.9%) (91), India. The use of different indicators for diabetes screening in other studies in Southern India (85, 86, 88, 89, 94–96), including fasting blood glucose ( $\geq 126$  mg/dL; 1.1–12.4%), which measures different aspects of glucose metabolism, constrains the comparability of findings (71).

### Vitamin B<sub>12</sub> and adiposity.

In the current study, lower vitamin B<sub>12</sub> status was associated with increased central adiposity (elevated WC, WHR, TF%), overweight, and elevated WF%. To date, observational studies on vitamin B<sub>12</sub> and adiposity have largely focused on BMI, and findings have been heterogeneous. Lower total vitamin B<sub>12</sub> status (continuous or  $< 150$  pmol/L) was associated with higher BMI in adult women with a high prevalence of vitamin B<sub>12</sub> deficiency ( $< 148$  or  $< 150$  pmol/L; 20–50%) in India and Turkey (97, 98). In clinical (23, 26, 99, 100) and population-based studies (38, 39) in adult men and women in India, findings have been heterogeneous: vitamin B<sub>12</sub>  $< 140$  pmol/L was associated with elevated WC but

not overweight (n=1,500) (38), and vitamin B<sub>12</sub> concentrations were not associated with overweight, or elevated WC or WHR (n=1,374) (39).

In population-based studies in adults in settings with lower prevalence of vitamin B<sub>12</sub> deficiency (Vietnam, U.S., Canada, Denmark), findings have been heterogeneous: lower vitamin B<sub>12</sub> status was associated with higher BMI, WC, WF% (37), and odds of obesity (41, 101) in some studies, but not others (40, 43). In analyses of U.S. NHANES data, lower vitamin B<sub>12</sub> quartiles were associated with higher odds of obesity in men and women (2011–2014) (41), while overweight was not associated with odds of vitamin B<sub>12</sub> <221 pmol/L in premenopausal women (BMI ≥18 kg/m<sup>2</sup>; 1988–1994) (43). Population characteristics (e.g., vitamin B<sub>12</sub> status, sex, genetics (37, 102, 103)) may contribute to inconsistent findings. The prevalence of severe obesity is rising in the U.S. (104), and the prevalence of obesity is expected to double from 2020 to 2035 in low- and middle-income countries (78); additional population-based data across regions is important as global risk factors and burden change.

### **Vitamin B<sub>12</sub> and other metabolic characteristics.**

In the current study, vitamin B<sub>12</sub> status was not associated with HbA1c. Studies to date on vitamin B<sub>12</sub> status and HbA1c have primarily been conducted in clinical and obstetric populations in other settings (Peru, Poland, Israel, Saudi Arabia) with heterogeneous results: lower vitamin B<sub>12</sub> status was associated with higher HbA1c in some studies (22, 105, 106) but not others (107, 108). In studies in adults in India, lower vitamin B<sub>12</sub> status was associated with higher fasting glucose (n=75) (26) and insulin resistance (n=300) (23) in clinical populations; higher insulin resistance and risk of type 2 diabetes in postpartum women (n=795) (98); and higher odds of type 2 diabetes in a nested case-control study (n=1,500) (38). Lower vitamin B<sub>12</sub> status was not associated with fasting glucose (109) or insulin resistance in women in other settings (n=122–346) (97, 110). The lack of association in the current study and the heterogeneous results in the literature could be due to the use of different biomarkers (e.g., HbA1c vs. fasting glucose) and populations at different risk for developing diabetes (e.g., women of reproductive age vs. patients with metabolic syndrome or postpartum women). For example, HbA1c may be affected by factors outside of glucose metabolism, such as iron deficiency (111) which is prevalent in this population (>40%) (54). Approximately 50% of diabetes cases are undiagnosed globally, with the highest burden in India and other low- and middle-income countries (77); identifying risk factors could help inform screening strategies.

In the current study, vitamin B<sub>12</sub> status was not associated with blood pressure or hypertension. There is limited data on vitamin B<sub>12</sub> and hypertension, and findings are heterogeneous. In India, vitamin B<sub>12</sub> concentrations were lower in adults with hypertension compared to normotensive individuals (n=130) (99), but vitamin B<sub>12</sub> concentrations were not associated with blood pressure (100) or hypertension (23) in other clinical populations (n=300–321). Lower vitamin B<sub>12</sub> status has been associated with other cardiovascular outcomes, including higher triglycerides (23, 26, 100, 109, 112), which were not evaluated in this study.

### Vitamin B<sub>12</sub> biomarkers/cut-offs.

Women with vitamin B<sub>12</sub> concentrations <221 pmol/L had increased adiposity, compared to ≥221 pmol/L, while results did not differ between women with vitamin B<sub>12</sub> concentrations ≥148-<221 pmol/L compared to women with concentrations ≥221 pmol/L. Analyses were constrained by the substantial proportion of women with vitamin B<sub>12</sub> concentrations <148 pmol/L (48.3%) compared to ≥148-<221 pmol/L (26.1%). In the U.S., the prevalence of vitamin B<sub>12</sub> ≥148-<221 pmol/L is more common than vitamin B<sub>12</sub> <148 pmol/L among non-pregnant, non-lactating women (11% vs. 2%) (1, 113, 114), as well as in non-pregnant women in other countries, including Canada (20–79 y; 20% vs. 6%) (101), the UK (19–39 y; 44% 150–258 pmol/L vs. 4% <150 pmol/L) (115), Ecuador (12–49 y; 20% vs. 4%) (116), Belize (15–49 y; 33% vs. 17%) (117), Guatemala (15–49 y; 30 % vs. 20%) (118), Columbia (18–49 y; 18.9% vs. 18.5%) (119), Malawi (15–49 y; 30% vs. 12%) (120), and Ghana (15–49 y; 12% vs. 7%) (121). Although results are similar to analyses conducted in the U.S. (41), additional data is needed to inform the role of vitamin B<sub>12</sub> in metabolic health in the context of concentrations ≥148-<221 pmol/L, given its global prevalence.

Both circulating and functional biomarkers are recommended for assessing vitamin B<sub>12</sub> status (3, 57, 58). Findings for impaired vitamin B<sub>12</sub> status (vitamin B<sub>12</sub><148 pmol/L and MMA>0.26 μmol) were similar to vitamin B<sub>12</sub> <148 pmol/L, and demonstrate a robust association between vitamin B<sub>12</sub> <148 pmol/L and adiposity in this population. Vitamin B<sub>12</sub> metabolism is closely linked to other nutrients, such as folate (60). Lower serum or plasma folate and higher RBC folate concentrations have been reported in individuals with overweight and obesity (122–127). In the current analysis, findings did not vary by RBC or serum folate status, consistent with other studies (98, 128) and with data suggesting that underlying conditions such as poor renal function and vitamin B<sub>12</sub> malabsorption may explain adverse health outcomes related to the combination of high folate and low vitamin B<sub>12</sub> status (129, 130).

### Strengths and limitations.

This population-based study is among the largest to date to examine associations of vitamin B<sub>12</sub> with metabolic characteristics in women. This analysis included circulating and functional biomarkers of vitamin B<sub>12</sub> status, and comprehensive assessment of body composition—in a population with a high prevalence of vitamin B<sub>12</sub> concentrations <148 pmol/L and adverse metabolic characteristics. This study has several limitations. The cross-sectional design does not inform temporal or causal relationships or changes over time. The limited range of concentrations within the lower quality control is a limitation of the total vitamin B<sub>12</sub> assay. Non-fasted archived blood samples constrained the analysis of other indicators of glycemic control. Although several potential confounders were evaluated, findings may be influenced by unmeasured or residual confounders. The response rate in the parent study (54%) is comparable to other population-based biomarker surveys (131); although participants were similar to non-participants (48), unmeasured factors may have influenced participation.

## CONCLUSIONS

In summary, the prevalence of vitamin B<sub>12</sub> <148 pmol/L and adverse metabolic characteristics was substantial in this population, including one-third of women with overweight, 21% with elevated waist-hip ratio ( $\geq 0.85$ ), and one-fourth with elevated HbA1c ( $\geq 5.7\%$ ). Lower vitamin B<sub>12</sub> status was associated with indicators of increased central adiposity and overweight. Evaluating the role of vitamin B<sub>12</sub> in the development of metabolic outcomes prospectively could inform screening and interventions to improve women's health in this population.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations Used:

|              |                                                 |
|--------------|-------------------------------------------------|
| <b>AMC</b>   | Arogyavaram Medical Centre                      |
| <b>BIA</b>   | Bioelectrical impedance analysis                |
| <b>BMI</b>   | body mass index                                 |
| <b>CDC</b>   | U.S. Centers for Disease Control and Prevention |
| <b>CI</b>    | confidence interval                             |
| <b>DRDC</b>  | Disability Research and Dissemination Center    |
| <b>GM</b>    | geometric mean                                  |
| <b>Hb</b>    | hemoglobin                                      |
| <b>HbA1c</b> | glycated hemoglobin                             |
| <b>LOD</b>   | limit of detection                              |
| <b>NIH</b>   | National Institutes of Health                   |
| <b>RBC</b>   | red blood cell                                  |

|            |                             |
|------------|-----------------------------|
| <b>TF%</b> | trunk percent fat mass      |
| <b>WF%</b> | whole body percent fat mass |
| <b>WC</b>  | waist circumference         |
| <b>WHO</b> | World Health Organization   |
| <b>WHR</b> | waist-hip ratio             |

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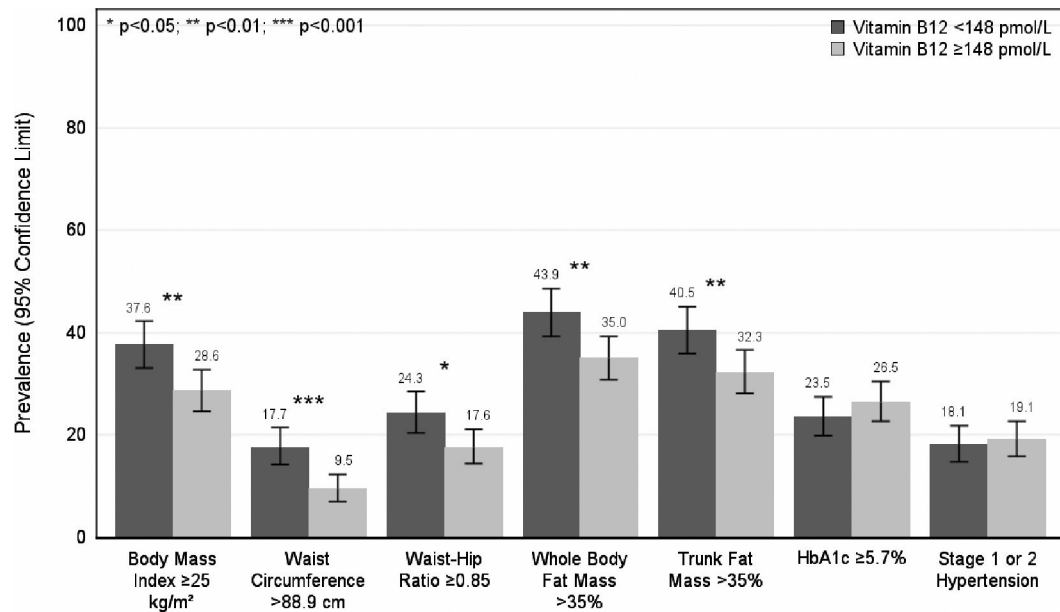


**Figure 1. Categorical metabolic characteristics by vitamin B<sub>12</sub> <148 pmol/L**

Values reported are n (%). Chi-square tests were used to evaluate differences in categorical metabolic characteristics by vitamin B<sub>12</sub> strata (vitamin B<sub>12</sub> <148 vs. ≥148 pmol/L). Vitamin B<sub>12</sub> results outside assay limits of detection (LOD) were set to half the LOD (if below LOD, n=4) or 2-times LOD (if above LOD, n=6). Categorical body mass index, waist circumference, waist-hip ratio, whole body fat mass, and trunk fat mass measurements are among adults (≥18 y), and HbA1c and blood pressure measurements are among all participants (≥15 y). Abbreviations: HbA1c: glycosylated hemoglobin

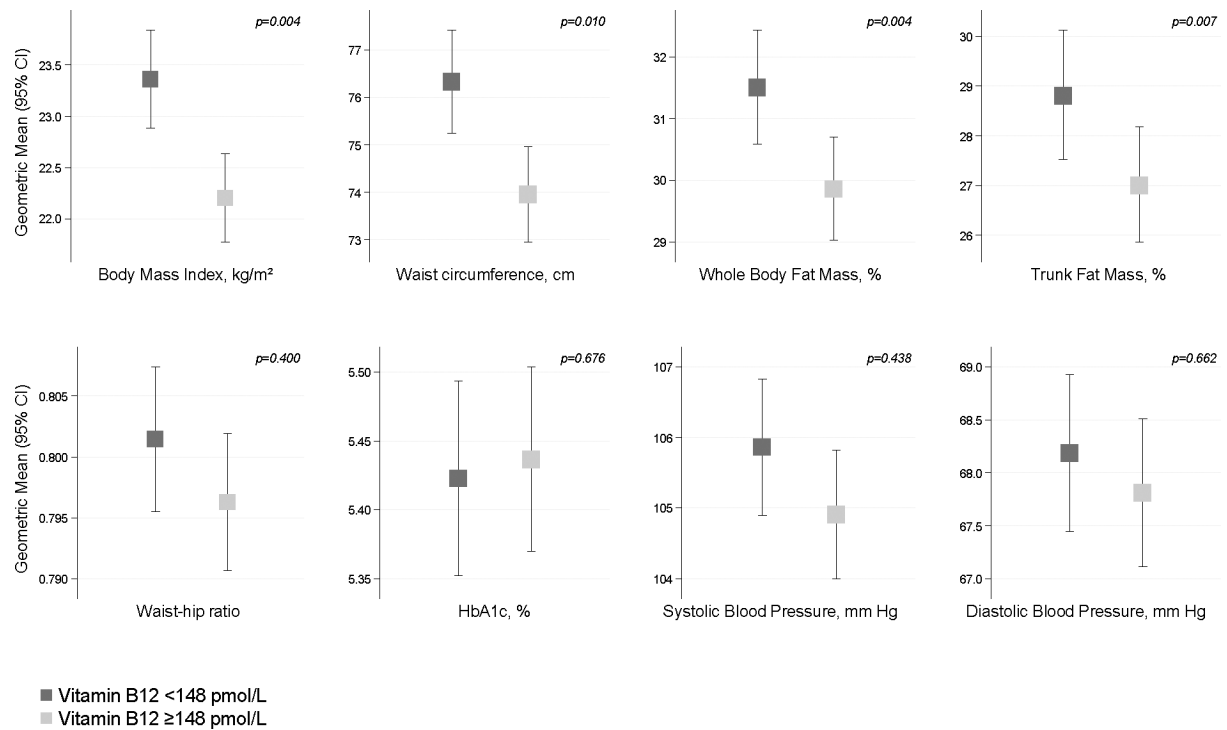


**Figure 2. Continuous metabolic characteristics by vitamin B<sub>12</sub> <148 pmol/L**

Values reported are geometric means (95% CI). One-way ANOVA was used to evaluate differences in continuous metabolic characteristics by vitamin B<sub>12</sub> strata (vitamin B<sub>12</sub> <148 vs. ≥148 pmol/L). Vitamin B<sub>12</sub> results outside assay limits of detection (LOD) were set to half the LOD (if below LOD, n=4) or 2-times LOD (if above LOD, n=6). HbA1c was natural logarithmically transformed prior to analyses. Continuous whole body fat mass and trunk fat mass measurements are among adults (≥18 y), and body mass index, waist circumference, waist-hip ratio, HbA1c, and blood pressure measurements are among all participants (≥15 y). Abbreviations: HbA1c: glycosylated hemoglobin, 95% CI: 95% confidence interval

Table 1.

Sociodemographic characteristics of the study population<sup>1</sup>

|                                                     | Total |                         | Rural (n=788)           |                         | Urban (n=192)        |                      |
|-----------------------------------------------------|-------|-------------------------|-------------------------|-------------------------|----------------------|----------------------|
|                                                     | n     | n (%) or GM (95% CI)    | n (%) or GM (95% CI)    | n (%) or GM (95% CI)    | n (%) or GM (95% CI) | n (%) or GM (95% CI) |
| <b>Sociodemographic</b>                             |       |                         |                         |                         |                      |                      |
| Household size                                      | 980   | 4.5 (4.4, 4.7)          | 4.6 (4.4, 4.7)          | 4.4 (4.1, 4.7)          |                      |                      |
| Monthly household income, INR <sup>2</sup>          | 979   | 5452.1 (5260.9, 5650.2) | 5474.3 (5260.7, 5696.6) | 5361.8 (4946.6, 5811.8) |                      |                      |
| Age, y                                              | 980   | 28.8 (28.4, 29.3)       | 28.8 (28.3, 29.3)       | 29.1 (28.1, 30.2)       |                      |                      |
| 15 to <18                                           |       | 49 (5.0)                | 41 (5.2)                | 8 (4.2)                 |                      |                      |
| 18 to <26                                           |       | 229 (23.4)              | 187 (23.7)              | 42 (21.9)               |                      |                      |
| 26 to <36                                           |       | 453 (46.2)              | 359 (45.6)              | 94 (49.0)               |                      |                      |
| 36 to 40                                            |       | 249 (25.4)              | 201 (25.5)              | 48 (25.0)               |                      |                      |
| Highest level of education completed                | 975   |                         |                         |                         |                      |                      |
| No formal schooling                                 |       | 158 (16.2)              | 125 (15.9)              | 33 (17.3)               |                      |                      |
| Primary (grades 1–8)                                |       | 351 (36.0)              | 280 (35.7)              | 71 (37.2)               |                      |                      |
| Secondary (grades 9–12), college or graduate degree |       | 466 (47.8)              | 379 (48.3)              | 87 (45.5)               |                      |                      |
| Employment status                                   | 952   |                         |                         |                         |                      |                      |
| Not formally employed                               |       | 476 (50.0)              | 384 (50.3)              | 92 (48.9)               |                      |                      |
| Part time or seasonal                               |       | 92 (9.7)                | 72 (9.4)                | 20 (10.6)               |                      |                      |
| Full time                                           |       | 282 (29.6)              | 222 (29.1)              | 60 (31.9)               |                      |                      |
| Student                                             |       | 102 (10.7)              | 86 (11.3)               | 16 (8.5)                |                      |                      |
| Marital status                                      | 975   |                         |                         |                         |                      |                      |
| Currently married                                   |       | 774 (79.4)              | 617 (78.7)              | 157 (82.2)              |                      |                      |
| Widowed, divorced, living separately                |       | 35 (3.6)                | 29 (3.7)                | 6 (3.1)                 |                      |                      |
| Never married                                       |       | 166 (17.0)              | 138 (17.6)              | 28 (14.7)               |                      |                      |
| Parity                                              | 975   |                         |                         |                         |                      |                      |
| Nulliparous                                         |       | 231 (23.7)              | 187 (23.9)              | 44 (23.0)               |                      |                      |
| Primiparous                                         |       | 90 (9.2)                | 68 (8.7)                | 22 (11.5)               |                      |                      |
| Multiparous                                         |       | 654 (67.1)              | 529 (67.5)              | 125 (65.4)              |                      |                      |
| Gravidity                                           | 975   |                         |                         |                         |                      |                      |
| 0                                                   |       | 207 (21.2)              | 169 (21.6)              | 38 (19.9)               |                      |                      |

|                                             | Total |                      | Rural (n=788)        |                      | Urban (n=192)        |                      |
|---------------------------------------------|-------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                                             | n     | n (%) or GM (95% CI) | n (%) or GM (95% CI) | n (%) or GM (95% CI) | n (%) or GM (95% CI) | n (%) or GM (95% CI) |
| 1                                           |       | 69 (7.1)             | 54 (6.9)             | 15 (7.9)             |                      |                      |
| ≥2                                          |       | 699 (71.7)           | 561 (71.6)           | 138 (72.3)           |                      |                      |
| Dietary preference <sup>2</sup>             | 974   |                      |                      |                      |                      |                      |
| Vegan                                       |       | 3 (0.3)              | 3 (0.4)              | 0 (0.0)              |                      |                      |
| Vegetarian                                  |       | 64 (6.6)             | 63 (8.0)             | 1 (0.5)              |                      |                      |
| Non-vegetarian                              |       | 907 (93.1)           | 717 (91.6)           | 190 (99.5)           |                      |                      |
| Frequency of animal-source food consumption |       |                      |                      |                      |                      |                      |
| Dairy                                       | 974   |                      |                      |                      |                      |                      |
| Never                                       |       | 53 (5.4)             | 42 (5.4)             | 11 (5.8)             |                      |                      |
| Almost never (<1x/mo)                       |       | 35 (3.6)             | 35 (4.5)             | 0 (0.0)              |                      |                      |
| Occasionally (<1x/wk)                       |       | 79 (8.1)             | 63 (8.0)             | 16 (8.4)             |                      |                      |
| Often (~1x/wk)                              |       | 152 (15.6)           | 114 (14.6)           | 38 (19.9)            |                      |                      |
| Very often (>1x/wk)                         |       | 655 (67.2)           | 529 (67.6)           | 126 (66.0)           |                      |                      |
| Egg                                         | 969   |                      |                      |                      |                      |                      |
| Never                                       |       | 72 (7.4)             | 65 (8.4)             | 7 (3.7)              |                      |                      |
| Almost never (<1x/mo)                       |       | 107 (11.0)           | 95 (12.2)            | 12 (6.3)             |                      |                      |
| Occasionally (<1x/wk)                       |       | 178 (18.4)           | 134 (17.2)           | 44 (23.0)            |                      |                      |
| Often (~1x/wk)                              |       | 376 (38.8)           | 289 (37.1)           | 87 (45.5)            |                      |                      |
| Very often (>1x/wk)                         |       | 236 (24.4)           | 195 (25.1)           | 41 (21.5)            |                      |                      |
| Poultry                                     | 973   |                      |                      |                      |                      |                      |
| Never                                       |       | 106 (10.9)           | 99 (12.7)            | 7 (3.7)              |                      |                      |
| Almost never (<1x/mo)                       |       | 81 (8.3)             | 67 (8.6)             | 14 (7.3)             |                      |                      |
| Occasionally (<1x/wk)                       |       | 119 (12.2)           | 100 (12.8)           | 19 (9.9)             |                      |                      |
| Often (~1x/wk)                              |       | 537 (55.2)           | 409 (52.3)           | 128 (67.0)           |                      |                      |
| Very often (>1x/wk)                         |       | 130 (13.4)           | 107 (13.7)           | 23 (12.0)            |                      |                      |
| Meat                                        | 973   |                      |                      |                      |                      |                      |
| Never                                       |       | 219 (22.5)           | 197 (25.2)           | 22 (11.5)            |                      |                      |
| Almost never (<1x/mo)                       |       | 303 (31.1)           | 240 (30.7)           | 63 (33.0)            |                      |                      |
| Occasionally (<1x/wk)                       |       | 213 (21.9)           | 148 (18.9)           | 65 (34.0)            |                      |                      |

|                               | Total                | Rural (n=788)        | Urban (n=192)        |
|-------------------------------|----------------------|----------------------|----------------------|
|                               | n (%) or GM (95% CI) | n (%) or GM (95% CI) | n (%) or GM (95% CI) |
| Often (~1x/wk)                | 209 (21.5)           | 169 (21.6)           | 40 (20.9)            |
| Very often (>1x/wk)           | 29 (3.0)             | 28 (3.6)             | 1 (0.5)              |
| Fish                          | 973                  |                      |                      |
| Never                         | 308 (31.7)           | 261 (33.4)           | 47 (24.6)            |
| Almost never (<1x/mo)         | 388 (39.9)           | 298 (38.1)           | 90 (47.1)            |
| Occasionally (<1x/wk)         | 178 (18.3)           | 139 (17.8)           | 39 (20.4)            |
| Often (~1x/wk)                | 74 (7.6)             | 61 (7.8)             | 13 (6.8)             |
| Very often (>1x/wk)           | 25 (2.6)             | 23 (2.9)             | 2 (1.0)              |
| Ever smoker                   | 81 (8.3)             | 70 (8.9)             | 11 (5.8)             |
| Ever diagnosed with diabetes  | 17 (1.8)             | 7 (0.9)              | 10 (5.7)             |
| Ever diagnosed with diabetes  | 974                  |                      |                      |
| Ever diagnosed with diabetes  | 930                  |                      |                      |
| Ever diagnosed with diabetes  | 17                   |                      |                      |
| Type 2 diabetes mellitus      | 8 (47.1)             | 3 (42.9)             | 5 (50.0)             |
| Type 1 diabetes mellitus      | 2 (11.8)             | 1 (14.3)             | 1 (10.0)             |
| Gestational diabetes mellitus | 2 (11.8)             | 0 (0.0)              | 2 (20.0)             |
| Don't know                    | 5 (29.4)             | 3 (42.9)             | 2 (20.0)             |

<sup>1</sup>Values reported are geometric means (95% Confidence Interval) and n (%)

<sup>2</sup>1 USD ≈ 70 INR

<sup>3</sup>Vegetarian: consumed milk and/or eggs; Nonvegetarian: consumed poultry, meat, and/or fish  
Abbreviations: GM: geometric mean, INR: Indian rupees, USD: United States Dollar, 95% CI: 95% confidence interval

Table 2.

Metabolic characteristics of the study population<sup>2</sup>

|                                    | Total |                      |     | Rural                |     |                      | Urban |  |  | p-value <sup>5</sup> |
|------------------------------------|-------|----------------------|-----|----------------------|-----|----------------------|-------|--|--|----------------------|
|                                    | n     | n (%) or GM (95% CI) | n   | n (%) or GM (95% CI) | n   | n (%) or GM (95% CI) |       |  |  |                      |
| Weight, kg                         | 969   | 53.0 (52.2, 53.7)    | 778 | 52.6 (51.7, 53.4)    | 191 | 54.6 (52.8, 56.4)    | 0.06  |  |  |                      |
| Height, cm                         | 969   | 153.2 (152.9, 153.6) | 778 | 153.4 (153.0, 153.8) | 191 | 152.7 (151.9, 153.5) | 0.14  |  |  |                      |
| Body mass index, kg/m <sup>2</sup> | 969   | 22.6 (22.2, 22.9)    | 778 | 22.3 (22.0, 22.7)    | 191 | 23.4 (22.7, 24.2)    | 0.01  |  |  |                      |
| <18.5 <i>I, 3</i>                  | 920   | 177 (19.2)           | 737 | 153 (20.8)           | 183 | 24 (13.1)            | 0.02  |  |  |                      |
| 18.5 to <25.0 <i>I, 3</i>          |       | 440 (47.8)           |     | 354 (48.0)           |     | 86 (47.0)            |       |  |  |                      |
| 25.0 to <30.0 <i>I, 3</i>          |       | 215 (23.4)           |     | 159 (21.6)           |     | 56 (30.6)            |       |  |  |                      |
| ≥30.0 <i>I, 3</i>                  |       | 88 (9.6)             |     | 71 (9.6)             |     | 17 (9.3)             |       |  |  |                      |
| Waist circumference, cm            | 969   | 74.6 (73.9, 75.3)    | 778 | 74.2 (73.4, 75.0)    | 191 | 76.3 (74.7, 78.0)    | 0.03  |  |  |                      |
| ≥88.9 <i>I</i>                     | 920   | 123 (13.4)           | 737 | 93 (12.6)            | 183 | 30 (16.4)            | 0.18  |  |  |                      |
| Hip circumference, cm              | 969   | 93.6 (93.0, 94.3)    | 778 | 93.1 (92.4, 93.8)    | 191 | 95.6 (94.2, 97.1)    | 0.004 |  |  |                      |
| Waist-hip ratio                    | 969   | 0.80 (0.79, 0.80)    | 778 | 0.80 (0.79, 0.80)    | 191 | 0.80 (0.79, 0.81)    | 0.78  |  |  |                      |
| ≥0.85 <i>I</i>                     | 920   | 191 (20.8)           | 737 | 151 (20.5)           | 183 | 40 (21.9)            | 0.68  |  |  |                      |
| Mid-upper arm circumference, cm    | 969   | 26.7 (26.5, 27.0)    | 778 | 26.6 (26.3, 26.9)    | 191 | 27.3 (26.7, 27.9)    | 0.03  |  |  |                      |
| Triceps skinfold thickness, mm     | 968   | 18.8 (18.4, 19.3)    | 777 | 19.0 (18.5, 19.5)    | 191 | 18.2 (17.3, 19.1)    | 0.09  |  |  |                      |
| Whole body, % fat mass <i>I</i>    | 922   | 30.6 (30.0, 31.2)    | 740 | 30.3 (29.6, 31.0)    | 182 | 32.0 (30.6, 33.5)    | 0.04  |  |  |                      |
| >35% <i>I</i>                      |       | 362 (39.3)           |     | 280 (37.8)           |     | 82 (45.1)            | 0.07  |  |  |                      |
| Trunk, % fat mass <i>I</i>         | 922   | 27.8 (27.0, 28.7)    | 740 | 27.5 (26.6, 28.5)    | 182 | 29.1 (27.1, 31.2)    | 0.09  |  |  |                      |
| >35% <i>I</i>                      |       | 334 (36.2)           |     | 259 (35.0)           |     | 75 (41.2)            | 0.12  |  |  |                      |
| HbA1c, %                           | 979   | 5.4 (5.4, 5.5)       | 787 | 5.4 (5.4, 5.5)       | 192 | 5.5 (5.4, 5.6)       | 0.08  |  |  |                      |
| <5.7                               |       | 734 (75.0)           |     | 595 (75.6)           |     | 139 (72.4)           | 0.14  |  |  |                      |
| ≥5.7 to <6.5                       |       | 196 (20.0)           |     | 158 (20.1)           |     | 38 (19.8)            |       |  |  |                      |
| ≥6.5                               |       | 49 (5.0)             |     | 34 (4.3)             |     | 15 (7.8)             |       |  |  |                      |
| ≥5.7                               |       | 245 (25.0)           |     | 192 (24.4)           |     | 53 (27.6)            | 0.36  |  |  |                      |
| SBP, mmHg                          | 945   | 104.9 (104.3, 105.6) | 754 | 105.0 (104.3, 105.8) | 191 | 104.6 (103.1, 106.0) | 0.57  |  |  |                      |
| DBP, mmHg                          | 945   | 67.7 (67.2, 68.2)    | 754 | 67.8 (67.2, 68.3)    | 191 | 67.6 (66.6, 68.7)    | 0.86  |  |  |                      |

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|                             | Total |                      |     | Rural                |     |                      | Urban |                      |      | p-value <sup>5</sup> |
|-----------------------------|-------|----------------------|-----|----------------------|-----|----------------------|-------|----------------------|------|----------------------|
|                             | n     | n (%) or GM (95% CI) | n   | n (%) or GM (95% CI) | n   | n (%) or GM (95% CI) | n     | n (%) or GM (95% CI) |      |                      |
| Blood pressure <sup>4</sup> | 945   |                      | 754 |                      | 191 |                      |       |                      | 0.85 |                      |
| Normal                      |       | 741 (78.4)           |     | 593 (78.6)           |     | 148 (77.5)           |       |                      |      |                      |
| Elevated                    |       | 28 (3.0)             |     | 22 (2.9)             |     | 6 (3.1)              |       |                      |      |                      |
| Stage 1 HTN                 |       | 155 (16.4)           |     | 121 (16.0)           |     | 34 (17.8)            |       |                      |      |                      |
| Stage 2 HTN                 |       | 21 (2.2)             |     | 18 (2.4)             |     | 3 (1.6)              |       |                      |      |                      |
| Stage 1 or 2 HTN            |       | 176 (18.6)           |     | 139 (18.4)           |     | 37 (19.4)            |       |                      | 0.77 |                      |

<sup>1</sup> Among women ≥18 years old

<sup>2</sup> Values reported are geometric means (GM) and 95% Confidence Interval and n (%)

<sup>3</sup> BMI categories as defined by the WHO (62)

<sup>4</sup> Blood pressure was categorized as normal (SBP <120 and DBP <80 mmHg), elevated blood pressure (SBP 120–129 and DBP <80 mmHg), stage 1 hypertension (SBP 130–139 or DBP 80–89 mmHg), and stage 2 hypertension (SBP ≥140 or DBP ≥90 mmHg) (70)

<sup>5</sup> Chi-square statistics and one-way ANOVA were used to evaluate differences in categorical and continuous variables, respectively; HbA1c was natural logarithmically transformed prior to analyses

Abbreviations: BMI, body mass index, DBP: diastolic blood pressure, GM: geometric mean, HbA1c: glycosylated hemoglobin, HTN: hypertension, SBP: systolic blood pressure, 95% CI: 95% confidence interval

**Table 3**  
Associations between total vitamin B<sub>12</sub> (exposure) and metabolic characteristics in women of reproductive age

| Characteristics                     | n   | Univariate            |                      |                                 | Multivariable        |  |  |
|-------------------------------------|-----|-----------------------|----------------------|---------------------------------|----------------------|--|--|
|                                     |     | P (SE) or PR (95% CI) | p-value <sup>2</sup> | P (SE) or PR (95% CI)           | p-value <sup>2</sup> |  |  |
| Body mass index, kg/m <sup>2</sup>  | 967 | -0.65 (0.28)          | 0.021                | -0.92 (0.27) <sup>3</sup>       | 0.001                |  |  |
| ≥25.0 <sup>1</sup>                  | 918 | 0.86 (0.74, 1.01)     | 0.064                | 0.82 (0.71, 0.96) <sup>4</sup>  | 0.013                |  |  |
| Waist circumference, cm             | 967 | -1.18 (0.64)          | 0.065                | -1.64 (0.61) <sup>5</sup>       | 0.007                |  |  |
| >88.9 <sup>1</sup>                  | 918 | 0.64 (0.49, 0.85)     | 0.002                | 0.64 (0.49, 0.85) <sup>6</sup>  | 0.002                |  |  |
| Waist-hip ratio                     | 967 | 0.001 (0.003)         | 0.678                | -0.001 (0.003) <sup>5</sup>     | 0.817                |  |  |
| ≥0.85 <sup>1</sup>                  | 918 | 0.86 (0.70, 1.07)     | 0.176                | 0.81 (0.66, 1.00) <sup>7</sup>  | 0.049                |  |  |
| Whole body, % fat mass <sup>1</sup> | 920 | -1.00 (0.50)          | 0.044                | -1.34 (0.49) <sup>8</sup>       | 0.007                |  |  |
| >35% <sup>1</sup>                   | 920 | 0.90 (0.79, 1.03)     | 0.125                | 0.86 (0.76, 0.98) <sup>9</sup>  | 0.028                |  |  |
| Trunk, % fat mass <sup>1</sup>      | 920 | -1.11 (0.61)          | 0.070                | -1.51 (0.61) <sup>8</sup>       | 0.013                |  |  |
| >35% <sup>1</sup>                   | 920 | 0.91 (0.79, 1.05)     | 0.209                | 0.88 (0.77, 1.01) <sup>5</sup>  | 0.065                |  |  |
| HbA1c, %                            | 978 | 0.01 (0.01)           | 0.213                | 0.004 (0.01) <sup>10</sup>      | 0.543                |  |  |
| ≥5.7                                | 978 | 1.19 (1.00, 1.41)     | 0.055                | 1.12 (0.95, 1.33) <sup>10</sup> | 0.199                |  |  |
| SBP, mmHg                           | 944 | -0.003 (0.56)         | 0.996                | -0.36 (0.55) <sup>5</sup>       | 0.515                |  |  |
| DBP, mmHg                           | 944 | 0.11 (0.43)           | 0.791                | -0.16 (0.42) <sup>5</sup>       | 0.704                |  |  |
| Stage 1 or 2 HTN                    | 944 | 1.04 (0.84, 1.29)     | 0.730                | 0.99 (0.81, 1.23) <sup>3</sup>  | 0.956                |  |  |

<sup>1</sup> Among women ≥18 years old

<sup>2</sup> Linear and binomial regression models; total vitamin B<sub>12</sub> and HbA1c were natural logarithmically transformed prior to analyses. Vitamin B<sub>12</sub> results outside assay limits of detection (LOD) were set to half the LOD (if below LOD, n=4) or 2-times LOD (if above LOD, n=6). In multivariable models, observations with missing data for covariates (<3%, i.e., education, n=5; marital status, n=5; gravidity, n=5; employment status, n=28) were retained in analyses using the missing indicator method, in which a dummy variable to indicate missing and a variable with missing values set to the reference value were included in the model (75)

Multivariable models adjusted for:

<sup>3</sup> age, currently married, gravidity, employment

<sup>4</sup> currently married, employment

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age, education, currently married, gravidity, employment  
5  
6  
7  
8  
9  
10  
No variables met criteria to be included as covariates  
education, employment  
currently married, gravidity, employment  
education, currently married, employment  
education, currently married, gravidity, employment

Abbreviations: DBP: diastolic blood pressure, HbA1c: glycated hemoglobin, HTN: hypertension, PR: prevalence ratio, SBP: systolic blood pressure, SE: standard error, 95% CI: 95% confidence interval

**Table 4.** Associations between vitamin B<sub>12</sub> <148 pmol/L (exposure) and metabolic characteristics in women of reproductive age

| Characteristics                     | N   | Univariate                  |                      |                                | Multivariable        |                                |                      |
|-------------------------------------|-----|-----------------------------|----------------------|--------------------------------|----------------------|--------------------------------|----------------------|
|                                     |     | $\beta$ (SE) or PR (95% CI) | p-value <sup>2</sup> | $\beta$ (SE) or PR (95% CI)    | p-value <sup>2</sup> | $\beta$ (SE) or PR (95% CI)    | p-value <sup>2</sup> |
| Body mass index, kg/m <sup>2</sup>  | 967 | 0.98 (0.34)                 | 0.004                | 1.29 (0.32) <sup>3</sup>       | <0.0001              | 1.29 (0.32) <sup>3</sup>       | <0.0001              |
| ≥25.01                              | 918 | 1.31 (1.09, 1.58)           | 0.004                | 1.31 (1.09, 1.58) <sup>4</sup> | 0.004                | 1.31 (1.09, 1.58) <sup>4</sup> | 0.004                |
| Waist circumference, cm             | 967 | 1.96 (0.76)                 | 0.010                | 2.65 (0.72) <sup>5</sup>       | <0.001               | 2.65 (0.72) <sup>5</sup>       | <0.001               |
| >88.9 <sup>1</sup>                  | 918 | 1.85 (1.32, 2.60)           | <0.001               | 1.85 (1.32, 2.60) <sup>4</sup> | <0.001               | 1.85 (1.32, 2.60) <sup>4</sup> | <0.001               |
| Waist-hip ratio                     | 967 | 0.003 (0.004)               | 0.400                | 0.01 (0.004) <sup>6</sup>      | 0.120                | 0.01 (0.004) <sup>6</sup>      | 0.120                |
| ≥0.85 <sup>1</sup>                  | 918 | 1.38 (1.07, 1.78)           | 0.013                | 1.38 (1.07, 1.78) <sup>4</sup> | 0.013                | 1.38 (1.07, 1.78) <sup>4</sup> | 0.013                |
| Whole body, % fat mass <sup>1</sup> | 920 | 1.72 (0.60)                 | 0.004                | 1.72 (0.60) <sup>4</sup>       | 0.004                | 1.72 (0.60) <sup>4</sup>       | 0.004                |
| >35% <sup>1</sup>                   | 920 | 1.26 (1.07, 1.48)           | 0.006                | 1.29 (1.10, 1.51) <sup>7</sup> | 0.001                | 1.29 (1.10, 1.51) <sup>7</sup> | 0.001                |
| Trunk, % fat mass <sup>1</sup>      | 920 | 1.99 (0.73)                 | 0.007                | 1.99 (0.73) <sup>4</sup>       | 0.007                | 1.99 (0.73) <sup>4</sup>       | 0.007                |
| >35% <sup>1</sup>                   | 920 | 1.25 (1.06, 1.49)           | 0.010                | 1.25 (1.06, 1.49) <sup>4</sup> | 0.010                | 1.25 (1.06, 1.49) <sup>4</sup> | 0.010                |
| HbA1c, %                            | 978 | -0.004 (0.01)               | 0.676                | 0.0005 (0.01) <sup>6</sup>     | 0.956                | 0.0005 (0.01) <sup>6</sup>     | 0.956                |
| ≥5.7                                | 978 | 0.89 (0.71, 1.10)           | 0.285                | 0.93 (0.75, 1.15) <sup>5</sup> | 0.498                | 0.93 (0.75, 1.15) <sup>5</sup> | 0.498                |
| SBP, mmHg                           | 944 | 0.53 (0.68)                 | 0.438                | 1.02 (0.66) <sup>6</sup>       | 0.122                | 1.02 (0.66) <sup>6</sup>       | 0.122                |
| DBP, mmHg                           | 944 | 0.23 (0.52)                 | 0.662                | 0.61 (0.50) <sup>6</sup>       | 0.222                | 0.61 (0.50) <sup>6</sup>       | 0.222                |
| Stage 1 or 2 HTN                    | 944 | 0.95 (0.73, 1.24)           | 0.689                | 1.02 (0.78, 1.32) <sup>5</sup> | 0.898                | 1.02 (0.78, 1.32) <sup>5</sup> | 0.898                |

<sup>1</sup> Among women ≥18 years old

<sup>2</sup> Linear and binomial regression models; HbA1c was natural logarithmically transformed prior to analyses. Vitamin B<sub>12</sub> results outside assay limits of detection (LOD) were set to half the LOD (if below LOD, n=4) or 2-times LOD (if above LOD, n=6). In multivariable models, observations with missing data for covariates (<3%, i.e., education, n=5; marital status, n=5; gravidity, n=5; employment status, n=28) were retained in analyses using the missing indicator method, in which a dummy variable to indicate missing and a variable with missing values set to the reference value were included in the model (75)

Multivariable models adjusted for:

<sup>3</sup> age, currently married, employment

<sup>4</sup> No variables met criteria to be included as covariates in multivariable models

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age, currently married, gravidity, employment  
age, education, currently married, gravidity, employment  
age

Abbreviations: DBP: diastolic blood pressure, HbA1c: glycated hemoglobin, HTN: hypertension, PR: prevalence ratio, SBP: systolic blood pressure, SE: standard error, 95% CI: 95% confidence interval

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**Table 5.**

Associations between impaired vitamin B<sub>12</sub> status (vitamin B<sub>12</sub> <148 pmol/L; exposure) and metabolic characteristics in women of reproductive age

| Characteristics                     | n   | Univariate                  |                      | Multivariable                  |                      |
|-------------------------------------|-----|-----------------------------|----------------------|--------------------------------|----------------------|
|                                     |     | $\beta$ (SE) or PR (95% CI) | p-value <sup>2</sup> | $\beta$ (SE) or PR (95% CI)    | p-value <sup>2</sup> |
| Body mass index, kg/m <sup>2</sup>  | 967 | 0.40 (0.35)                 | 0.252                | 0.73 (0.34) <sup>3</sup>       | 0.031                |
| ≥25.0 <sup>1</sup>                  | 918 | 1.16 (0.97, 1.40)           | 0.117                | 1.22 (1.02, 1.46) <sup>4</sup> | 0.032                |
| Waist circumference, cm             | 967 | 0.54 (0.78)                 | 0.492                | 1.23 (0.75) <sup>3</sup>       | 0.103                |
| >88.9 <sup>1</sup>                  | 918 | 1.43 (1.03, 1.99)           | 0.033                | 1.48 (1.07, 2.06) <sup>5</sup> | 0.020                |
| Waist-hip ratio                     | 967 | -0.004 (0.004)              | 0.285                | -0.001 (0.004) <sup>3</sup>    | 0.716                |
| ≥0.85 <sup>1</sup>                  | 918 | 1.13 (0.88, 1.47)           | 0.341                | 1.18 (0.92, 1.52) <sup>6</sup> | 0.196                |
| Whole body, % fat mass <sup>1</sup> | 920 | 0.97 (0.62)                 | 0.116                | 1.28 (0.61) <sup>7</sup>       | 0.036                |
| >35% <sup>1</sup>                   | 920 | 1.12 (0.95, 1.32)           | 0.181                | 1.17 (1.00, 1.37) <sup>8</sup> | 0.055                |
| Trunk, % fat mass <sup>1</sup>      | 920 | 1.11 (0.76)                 | 0.146                | 1.51 (0.76) <sup>5</sup>       | 0.046                |
| >35% <sup>1</sup>                   | 920 | 1.15 (0.97, 1.37)           | 0.121                | 1.20 (1.02, 1.42) <sup>8</sup> | 0.033                |
| HbA1c, %                            | 978 | -0.01 (0.01)                | 0.441                | -0.003 (0.01) <sup>3</sup>     | 0.760                |
| ≥5.7                                | 978 | 0.97 (0.78, 1.22)           | 0.797                | 1.01 (0.81, 1.27) <sup>3</sup> | 0.901                |
| SBP, mmHg                           | 944 | 0.16 (0.70)                 | 0.814                | 0.72 (0.68) <sup>3</sup>       | 0.289                |
| DBP, mmHg                           | 944 | 0.02 (0.53)                 | 0.969                | 0.45 (0.52) <sup>3</sup>       | 0.392                |
| Stage 1 or 2 HTN                    | 944 | 0.94 (0.71, 1.23)           | 0.636                | 0.99 (0.75, 1.30) <sup>9</sup> | 0.952                |

<sup>1</sup> Among women ≥18 years old

<sup>2</sup> Linear and binomial regression models; HbA1c was natural logarithmically transformed prior to analyses. Vitamin B<sub>12</sub> results outside assay limits of detection (LOD) were set to half the LOD (if below LOD, n=4) or 2-times LOD (if above LOD, n=6). In multivariable models, observations with missing data for covariates (<3%, i.e., education, n=5; marital status, n=5; gravidity, n=5; employment status, n=28) were retained in analyses using the missing indicator method, in which a dummy variable to indicate missing and a variable with missing values set to the reference value were included in the model (75)

Multivariable models adjusted for:

<sup>3</sup> age, education, currently married, gravidity, employment

<sup>4</sup> age, currently married, employment

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5 employment  
6 age, education, employment  
7 currently married, employment  
8 age, education, currently married, employment  
9 age, currently married, gravidity, employment  
Abbreviations: DBP: diastolic blood pressure, HbA1c: glycated hemoglobin, HTN: hypertension, MMA, methylmalonic acid, PR: prevalence ratio, SBP: systolic blood pressure, SE: standard error, 95% CI:  
95% confidence interval