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Placental and Cord Blood Telomere Length in Relation to Maternal Nutritional Status

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ABSTRACT

Background: The uterine environment may be important for the chromosomal telomere length (TL) at birth, which, in turn, influences disease susceptibility throughout life. However, little is known about the importance of specific nutritional factors.

Objectives: We assessed the impact of multiple maternal nutritional factors on TL in placenta and cord blood.

Methods: In a population-based mother–child cohort in northwestern Argentina, we measured maternal weight, BMI, body fat percentage (BFP), and several nutrients [selenium, magnesium, calcium, zinc, manganese, iodine, vitamin B-12, folate, 25-hydroxycholecalciferol (25(OH)D_3)], hemoglobin, and homocysteine in maternal whole blood, serum, plasma, or urine during pregnancy (mean gestational week 27). We measured the relative TL (rTL) in placenta ($n = 99$) and cord blood ($n = 98$) at delivery by real-time PCR. Associations were evaluated by multivariable-adjusted linear regression.

Results: The women's prepregnancy BMI (kg/m^2; mean ± SD: 23.7 ± 4.1), body weight (55.4 ± 9.9 kg), and BFP (29.9 ± 5.5%), but not height (153 ± 5.3 cm), were inversely associated with placental rTL ($P < 0.01$ for all), with ∼0.5 SD shorter rTL for an IQR increase in prepregnancy body weight, BMI, or BFP. Also, impedance-based BFP, but not lean body mass, in the third trimester was associated with shorter placental rTL. In addition, serum vitamin B-12 (232 ± 96 pmol/L) in pregnancy ($P = 0.038$), but not folate or homocysteine, was associated with shorter placental rTL (0.2 SD for an IQR increase). In contrast, plasma 25(OH)D_3 (46 ± 15 nmol/L) was positively associated with placental rTL ($P < 0.01$), which increased by 0.4 SD for an IQR increase in 25(OH)D_3. No clear associations of the studied maternal nutritional factors were found with cord blood rTL.

Conclusions: Maternal BMI, BFP, and vitamin B-12 were inversely associated, whereas 25(OH)D_3 was positively associated, with placental TL. No association was observed with cord blood TL. Future studies should elucidate the role of placental TL for child health. *J Nutr* 2020;150:2646–2655.

Keywords: body fat, body weight, vitamin B-12 status, early-life programming, maternal nutrition in pregnancy, nutrients, newborn, placenta, vitamin D status, 25(OH)D_3

Introduction

Epidemiological and clinical studies have provided convincing evidence of the importance of the developmental origins of health and disease, but the involved mechanisms are not yet entirely clarified. Telomeres, essential for maintaining genomic integrity, are nucleotide repeats at the end of each chromosome arm in eukaryotes (1, 2). They are shortened by each cell division, eventually leading to aging of tissues and functions (3). In highly proliferating tissues there is also a counteracting elongation process under the influence of telomerase, the main telomere-maintaining enzyme. The telomerase activity is strictly regulated in both placenta and embryo and appears to be important for successful fetal development (4, 5). Still, the telomeres have a certain degree of plasticity, and recent studies show that the uterine environment may influence fetal telomere length (TL) (6–8). This is important to study, since TL at birth is a main predictor for TL throughout life (6, 9, 10), and TL later in life is associated with the risk of several outcomes,

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Abbreviations used: BFM, body fat mass; BFP, body fat percentage; GW, gestational week; Hb, hemoglobin; *HBB*, hemoglobin β chain; ICP-MS, inductively coupled plasma mass spectrometry; LBM, lean body mass; LMP, last menstrual period; rTL, relative telomere length; TL, telomere length; 25(OH)D_3, 25-hydroxycholecalciferol.

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such as cancer and cardiovascular diseases (11, 12). There is emerging evidence that factors such as maternal stress (13, 14), inflammation (15), gestational diabetes (16), exposure to air pollution (17, 18), tobacco smoke (19), and toxic metals (20) may influence offspring TL.

Telomeres may also be susceptible to poor or unbalanced nutrition (21). Prepregnancy BMI has been found to be inversely associated with newborn TL (22), whereas maternal folate (7, 23), vitamin C (24), and vitamin D (25) have been associated with longer telomeres in newborns. The aim of the present study was to assess the potential impact of multiple markers of maternal nutritional status on TL in placenta and cord blood. We measured both placenta and cord blood TL for 3 reasons. These tissues have different lifespan and functions; therefore, telomeres of the placenta likely reflect changes from early to late pregnancy, whereas the cells in cord blood have a shorter lifespan. Second, they may differ in sensitivity to external or internal stressors. Last, they may have different predictability for health outcomes.

Methods

Study population

The study was based on a mother–child cohort in the Andean part of the Salta province in northwestern Argentina (26, 27). The study area (altitude of 3180–4070 m above sea level) included the main village of San Antonio de los Cobres, with ~5900 inhabitants, and 9 much smaller villages located 40–216 km away. With assistance of the antenatal care unit at the hospital in San Antonio de los Cobres, all pregnant women in the study area with an estimated delivery date between October 2012 and December 2013 were invited to participate in the study. Out of a total of 221 pregnant women, 194 were enrolled (**Supplemental Figure 1**). Of the 100 women with placenta and cord blood samples, we obtained placental TL for 99 women and cord blood TL for 98 women. More details about the study area, recruitment of the cohort, and loss to follow-up have been described elsewhere (27).

The study was performed in accordance with the Declaration of Helsinki and approved by the Ministry of Health, Salta, Argentina, and the regional ethical committee at Karolinska Institutet, Stockholm, Sweden (2012/2:7). Prior to recruitment, written informed consent was obtained from all women after oral and written explanation of the study details. For the few women <18 y of age, informed consent was also obtained from the caregiver.

Data and sample collection

The study was designed to see the pregnant women at least once during pregnancy, as early as possible. Because women entered the study at different gestational weeks, and the fact that the study area was very large with few transportation possibilities, we obtained 1–3 samples per woman. Out of all women with data on relative TL (rTL; $n = 99$), 10 women had 3 study visits during pregnancy, 67 women had 2, and 22 women had 1 visit. Fifteen women were investigated in the first trimester [mean gestational week (GW) 10.8], 54 women in the second trimester (mean GW 21.8), and 80 women in the third trimester (mean GW 33.5). At the first visit (mean GW 23.8), height was measured in a standardized way, and the pregnant women were interviewed about age, last menstrual period (LMP), prepregnancy weight, parity, family income, educational level, smoking, alcohol consumption, coca chewing, and personal and family history of diseases. At each visit, the women were asked about health problems encountered during pregnancy, and blood and urine samples were collected. Body weight was measured at each visit (HCG-210QM scale, accurate to 100 g; GA.MA Professional, Italy). Gestational age at birth was calculated by subtracting the date of LMP from the date of birth. In the few cases of missing LMP, the ultrasound-based estimation was used. We calculated prepregnancy BMI (kg/m^2) from height (meters) and prepregnancy body weight (kilograms), and lean body mass (LBM) using the equation by Watson and coworkers (28). Prepregnancy body fat mass (BFM; kilograms) was obtained by subtracting LBM from the total body weight. BFM during pregnancy was measured by bioelectrical impedance (Body Fat Monitor HBF-302; Omron Healthcare, Inc.) at each visit and body fat percentage (BFP; %) was calculated.

Maternal venous blood samples were collected in Trace Elements Sodium Heparin tubes and in Trace Elements Serum Clot Activator tubes (Vacuette; Greiner Bio-One International GmbH). Serum and plasma were fractionated by centrifugation in a standardized way, exactly 15 min after blood withdrawal. Blood for DNA extraction was collected in EDTA tubes (Vacuette K3EDTA; Greiner Bio-One International GmbH). Because of the large study area, with many women living far from the village primary health care clinic, it was not possible to obtain fasting blood or urine samples or to collect samples at a fixed time of the day. Midstream spot urine samples were collected in disposable trace element–free plastic cups and immediately transferred to 20 mL polyethylene bottles (Zinsser Analytic GmbH), and frozen at −20°C.

Whole placentas were collected at delivery and immediately frozen at −20°C. At the time of sample preparation, the placentas were thawed, and the amnion was removed. A biopsy of ~5 cm^3 was cut out ~5 cm from the umbilical cord insertion and 1.0–1.5 cm below the fetal membranes, so as to obtain homogenous samples from the placental villous parenchyma. Approximately 25 mg of the latter biopsy was used to extract DNA for TL analysis, after as much as possible of the maternal blood was removed by washing with sterile PBS. Previous studies showed no significant influence of sampling site, mode of delivery, or fetal sex on placental TL (29). Cord blood was collected in the same types of tubes and processed in the same way as the maternal blood samples. All samples, including placentas, were transported on ice to Karolinska Institutet, Stockholm, where they were stored at −80°C (urine samples at −20°C) until analysis.

Measurement of TL

We obtained high-quality DNA and successfully measured rTL in 99 placentas and 98 cord blood samples. DNA from cord blood was extracted using an E.Z.N.A. Blood DNA Mini kit (Omega Bio-Tek, Inc.) and from placentas using a QIAamp DNA Mini kit (Qiagen), as described previously (20). The rTL was measured as the ratio between the signal intensity of the telomere sequences and the signal intensity of a single-copy gene [hemoglobin β chain (*HBB*)], using real-time PCR (7900HT; Applied Biosystems) according to Yeates et al. (30). In short, PCR reactions for telomere and *HBB* were run on separate plates with respective settings. The master mix for the telomere PCR was prepared with telomere-specific primers (0.45 μM of each primer), PCR buffer (1×) (all PCR reagents from Life Technologies), 1.75 mM $MgCl_2$, 0.8 mM deoxyribonucleotide triphosphate, 0.3 mM SYBR Green, Rox (1×), and 0.5 U Platinum *Taq* polymerase. Master mix for *HBB* was prepared with *HBB* primers (0.40 μM for each primer) and KAPA SYBR FAST qPCR Kit Master Mix (1×) ABI Prism (Kapa Biosystems). One calibrator DNA was prepared by pooling DNA from randomly selected samples. The calibrator DNA was then serially diluted to produce concentrations of 0.31–20 ng/μL for the standard curve. The standard curve and a negative control were included in each run. All samples, DNA standards, and negative controls were run in triplicate. R^2 for each standard curve was >0.99. If the SDs of cycle threshold of the triplicate were >0.1, the deviated one would be excluded. The rTL was obtained through calculating the ratio of the telomere repeat product to the single-copy gene product (here *HBB*) for each individual. The rTL ratio is an arbitrary value.

Biomarkers of nutritional status

Trace element concentrations were determined using inductively coupled plasma–mass spectrometry (ICP-MS; Agilent 7700x; Agilent Technologies) with collision/reaction cell technology. Concentrations of the essential trace elements selenium, magnesium, calcium, zinc, and manganese were measured in maternal whole blood or serum, depending on the suitability as a biomarker. Aliquots (0.2 mL) of

blood or serum samples were diluted 1:25 with an alkali solution, as described previously (27, 31). Iodine in urine was measured by ICP-MS after dilution 1:10 with 0.1% ammonium hydroxide (NH_4OH 25% wt:wt; Suprapur®; Merck KGaA). The concentrations in urine were corrected for variation in urine dilution by adjustment to the mean osmolality (694 mOsm/kg), measured by a digital cryoscopic osmometer (Osmomat 030, Gonotec Gesellschaft für Meβ- und Regeltechnik).

Serum concentrations of folate, homocysteine, and vitamin B-12 and plasma concentrations of 25-hydroxycholecalciferol [25(OH)D_3] were measured at Clinical Chemistry, Lund University Hospital, Sweden, using standard clinical analytical methods. Vitamin B-12 and folate were measured by an electrochemiluminescence immunoassay based on ruthenium derivatives (ECLI; Roche Diagnostics), homocysteine was measured by an indirect enzymatic method measuring the absorbance of NAD^+, and all were subsequently measured using a Cobas® 6000/8000 analyzer system (Roche Diagnostics International Ltd). Plasma 25(OH)D_3 was measured using LC connected to a triple quadrupole mass spectrometer (26). Blood hemoglobin (Hb) concentrations were measured using HemoCue 201+ (HemoCue AB) directly after venous blood withdrawal.

Statistical methods

Statistical analyses were performed using Stata version 11.2 (StataCorp LP). P values <0.05 were considered statistically significant. We did not correct for multiple testing, because of the concern that it might increase the type II error (32). Instead, we evaluated the consistency of associations and the effect size and compared the results against previous findings. Bivariate associations between nutrition markers and rTL were initially assessed by Spearman's rank correlation test (r_S) and visual evaluation of scatterplots. Differences between groups were tested by nonparametric Mann-Whitney U test or Kruskal-Wallis test.

Multivariable-adjusted linear regression analysis was performed to evaluate the associations between nutrition markers and rTL in placenta and cord blood. The crude model (model 1) included the nutrition marker only. The adjusted model (model 2) for placental rTL included factors known to affect TL: maternal age, education, prepregnancy BMI, and gestational age at birth. In the models with 25(OH)D_3, we additionally adjusted for season of sampling (summer/fall/spring/winter). We then made a model 3 for placental rTL with additional adjustment for the other nutritional factors that showed an association in model 2—that is, vitamin B-12 and 25(OH)D_3 (including season of sampling). Pre-eclampsia has been associated with shorter trophoblast TL (5); however, this was not included as covariate, as none of the women with placental rTL data reported having pre-eclampsia. For cord blood rTL, model 2 included adjustment for maternal age, GW at birth, and birth weight, according to previous studies (33). None of the nutritional factors showed a direct association with cord blood rTL, but in an additional model we further adjusted model 2 for placental rTL. Because previous studies have indicated sex-specific interactions with newborn rTL (14), we tested for a multiplicative interaction between fetal sex and the different nutrition markers in the adjusted models and performed stratified analyses. We carried out sensitivity analysis (model 4) adjusting for paternal age instead of maternal age in the adjusted models. Also, we additionally adjusted the placental rTL in model 3 for placental weight (model 5).

Results

General characteristics and nutritional factors

The characteristics of the cohort are presented in **Table 1**. The participating women ranged in age from 14 to 41 y. Thirty-three percent were first-time mothers and 11% delivered preterm (GW <37). The women were mostly of indigenous origin, with 85% reportedly belonging to the Kolla community and some to the Atacama and Tastil communities. None of the women reported smoking during pregnancy, but 5 reported that they occasionally used alcohol, although very seldomly. The prepregnancy BMI of the women ranged from 16.9 to 36.0. The women were generally short (range: 143–166 cm), contributing to the rather high BMI values. Indeed, the mean height of the women with prepregnancy BMI ≥30 was only 150.6 cm ($n = 11$). The estimated prepregnancy BFM correlated strongly with the impedance-based BFM, measured during pregnancy (r_s for the second trimester was 0.81, $n = 46$; in the third trimester was 0.76, $n = 68$). The impedance-based BFM in the second trimester correlated strongly with BMI, based on measured weight and height at the same point in time ($r_s = 0.88$, $n = 90$).

Table 1 also shows that rTL was longer in cord blood (1.26 ± 0.12) than in placenta (0.77 ± 0.21) and that the interindividual variation in rTL was markedly smaller in cord blood (CV: 9.5%) than in placenta (CV: 27%). Still, cord blood rTL correlated moderately with placental rTL ($r_s = 0.35$, $P = 0.0005$; **Supplemental Figure 2**). Neither placental rTL nor cord blood rTL differed by newborn sex (mean ± SD: 0.76 ± 0.21 in male and 0.78 ± 0.20 in female placentas, 1.26 ± 0.12 in male and 1.28 ± 0.12 in female cord blood). Placental rTL (Table 1) correlated inversely with prepregnancy BMI ($P = 0.072$), body weight ($P = 0.038$), and BFP ($P = 0.052$) and Hb in the third trimester ($P = 0.005$) (**Figure 1**A, B, C, F), as well as positively with paternal age (**Supplemental Figure 3**). Only Hb correlated (inversely) with cord blood rTL (Table 1).

Associations of nutritional factors with rTL in placenta

In the linear regression analysis, prepregnancy BMI, body weight and BFP were all inversely associated with placental rTL (**Table 2**). The unadjusted estimates (model 1) were markedly strengthened by the multivariable adjustment (models 2 and 3). Prepregnancy BMI, weight, and BFP were not combined in the same model as they were highly correlated ($r_s = 0.87$–0.97). The fully adjusted models (model 3) explained 24–26% of the variation in placental rTL. Maternal age was positively associated with placental rTL ($\beta = 0.011$; 95% CI: 0.003, 0.019; $P = 0.006$), whereas the mothers' education and gestational week at delivery were inversely associated with placental rTL ($\beta = -0.015$; 95% CI: −0.028, −0.001; $P = 0.031$; and $\beta = -0.026$; 95% CI: −0.048, −0.004; $P = 0.021$, respectively). Using BFP in the third trimester (impedance-based) instead of prepregnancy BFP (based on Watson equation) gave a slightly stronger association ($\beta = -0.020$; 95% CI: −0.033, −0.007; $P = 0.004$; $n = 63$; model 3). In contrast, LBM in the third trimester was not associated with placental rTL ($\beta = -0.002$; 95% CI: −0.013, 0.009; $P = 0.70$), and the estimate was weaker compared with that with prepregnancy LBM (based on Watson equation, $\beta = -0.016$; 95% CI: −0.029, −0.003; $P = 0.017$). The interaction term sex × prepregnancy BMI, body weight, or BFP ($P = 0.30$, 0.38, and 0.20, respectively) did not indicate any difference in the associations by newborn sex. Adjusting model 3 for paternal age instead of maternal age did not change the associations (**Supplemental Table 1**, model 4). However, paternal age (16–57 y) was strongly significant in the models with a similar estimate as maternal age (model 4, BMI: $\beta = 0.011$; 95% CI: 0.006, 0.017; $P < 0.001$, $n = 74$; $R^2 = 0.35$). Further adjusting model 3 by placental weight did not change the associations (**Supplemental Table 2**, model 5).

In addition, serum vitamin B-12 was inversely associated with placental rTL, especially after the covariate adjustment (Table 2). Using paternal age instead of maternal age in this

TABLE 1 General characteristics of the studied mothers and newborns and Spearman's rank correlation (r_s) with rTL in placenta and cord blood[1]

			Correlation with placental rTL		Correlation with cord blood rTL	
	n	Characteristics	r_s	*P*	r_s	*P*
Placental rTL	99	0.77 ± 0.21	—		0.347	<0.001
Cord blood rTL	98	1.26 ± 0.12	0.347	<0.001	—	
Maternal age, y	94	24.1 ± 6.7	0.074	0.478	−0.002	0.982
Paternal age, y	86	27.6 ± 8.0	0.217	0.046	0.082	0.459
Prepregnancy BMI, kg/m^2	90	23.6 ± 4.1	−0.192	0.072	−0.011	0.919
Height, cm	93	153 ± 5.3	0.009	0.931	−0.072	0.495
Prepregnancy weight, kg	92	55.4 ± 9.9	−0.218	0.038	−0.059	0.583
Prepregnancy body fat, percentage	89	29.9 ± 5.6	−0.208	0.052	−0.004	0.974
Maternal education, y	93	8.6 ± 3.3	−0.120	0.256	−0.042	0.692
Parity, *n*	93	1 (0–12)	0.128	0.223	0.076	0.473
Hemoglobin,[2] g/L	79	135 ± 11.8	−0.317	0.005	−0.312	0.005
Serum folate,[3] nmol/L	86	25.7 ± 5.6	0.091	0.408	0.143	0.194
Serum vitamin B-12,[3] pmol/L	86	232 ± 96	−0.115	0.296	−0.013	0.909
Serum homocysteine,[3] μmol/L	86	5.4 ± 1.3	−0.042	0.702	−0.101	0.363
Plasma 25(OH)D_3,[3] nmol/L	88	45.7 ± 15.3	0.084	0.438	0.086	0.429
Serum selenium,[3] μg/L	89	84.9 ± 14.4	−0.085	0.431	0.159	0.142
Serum magnesium,[3] mg/L	89	18.1 ± 1.6	−0.180	0.092	−0.008	0.945
Serum calcium,[3] mg/L	89	88.5 ± 4.1	−0.202	0.060	−0.032	0.766
Whole blood zinc,[3] mg/L	88	6.1 ± 0.83	−0.074	0.494	−0.094	0.391
Whole blood manganese,[3] μg/L	88	21.0 ± 7.9	−0.047	0.667	−0.039	0.722
Urinary iodine, μg/L	89	103 (35–708)	−0.029	0.788	−0.032	0.767
Systolic blood pressure,[2] mm Hg	81	105 ± 13.5	0.008	0.940	−0.034	0.767
Diastolic blood pressure,[2] mm Hg	81	67.0 ± 10.9	−0.011	0.920	−0.158	0.162
Coca chewing, times/wk	93	1 (0–28)	0.128	0.225	0.035	0.739
Gestational week at birth	99	38.7 ± 1.9	−0.128	0.210	0.053	0.607
Birth weight, g	99	3068 ± 374	−0.046	0.653	−0.116	0.256
Season of birth, % by every 3 mo	100	31/20/30/19	0.082	0.417	−0.075	0.462
Placental weight, g	90	630 ± 107	0.150	0.159	−0.027	0.799

[1] Values are means ± SDs or medians (range) unless otherwise indicated. rTL, relative telomere length; 25(OH)D_3, 25-hydroxycholecalciferol.
[2] During the third trimester.
[3] During pregnancy (mean gestational week 27).

model weakened the association by almost 25% (Supplemental Table 1, model 4), but further adjustment by placental weight did not change the association (Supplemental Table 2, model 5). As the scatterplot (Figure 1D) indicated a nonlinear association (positive association at low vitamin B-12 concentrations), we performed a spline regression analysis with the knot at the indicated turning point of 0.175 nmol/L. This showed a stronger (~43%) inverse association above the knot ($\beta = -0.676$; 95% CI: −1.187, −0.164; $P = 0.010$; $n = 81$). This association was not influenced by additional adjustment for the other measured markers of one-carbon metabolism—that is, folate and homocysteine ($\beta = -0.661$; 95% CI: −1.189, −0.134; $P = 0.015$; $n = 81$). The *P* value for the interaction sex × serum vitamin B-12 was 0.043 (model 3), and stratifying the spline regression in model 3 by newborn sex showed markedly a stronger association with rTL in male placentas ($\beta = -1.036$; 95% CI: −1.719, −0.353; $P = 0.004$; $n = 45$) than in female placentas ($\beta = -0.264$; 95% CI: −1.219, 0.691; $P = 0.58$; $n = 36$), although the numbers became small. The differences are illustrated in **Supplemental Figure 4**. The inverse association between Hb in late gestation and placental rTL, which was significant in the unadjusted model (Figure 1F), weakened markedly by the adjustment and was no longer statistically significant (Table 2, models 2 and 3).

Plasma 25(OH)D_3, on the other hand, was positively associated with placental rTL (Figure 1E), and the estimate ($\beta = 0.0024$ in model 1) was markedly strengthened (~50%) by the additional adjustments (Table 2, models 2 and 3; $\beta = 0.0039$ and 0.0036, respectively). As the scatterplot (Figure 1E) indicated a nonlinear association, we conducted a spline regression analysis (model 3) with a knot at 40 nmol/L of plasma 25(OH)D_3 (indicated turning point in Figure 1E). This showed a modestly stronger positive association (~30%) above the knot ($\beta = 0.0047$; 95% CI: 0.0008, 0.0086; $P = 0.018$; $n = 81$). There was no significant interaction with sex ($P = 0.24$, linear regression). The estimate (linear regression) was slightly strengthened ($\beta = 0.0048$) by entering paternal age instead of maternal age (Supplemental Table 1, model 4), but there was no major change by further adjustment for placental weight ($\beta = 0.0034$; Supplemental Table 2, model 5).

Associations of nutritional factors with rTL in cord blood

None of the measured nutritional factors was associated with cord blood rTL in the multivariable-adjusted analysis including all newborns (**Table 3**). Further adjusting for placental rTL did not improve the associations (**Supplemental Table 3**). Using BFP in the third trimester instead of prepregnancy BFP did not markedly change the association with cord blood rTL ($\beta = -0.003$; 95% CI: −0.010, 0.005; $P = 0.48$; $n = 68$). The association between maternal serum folate concentrations and cord blood rTL appeared to be U-shaped in the scatterplot

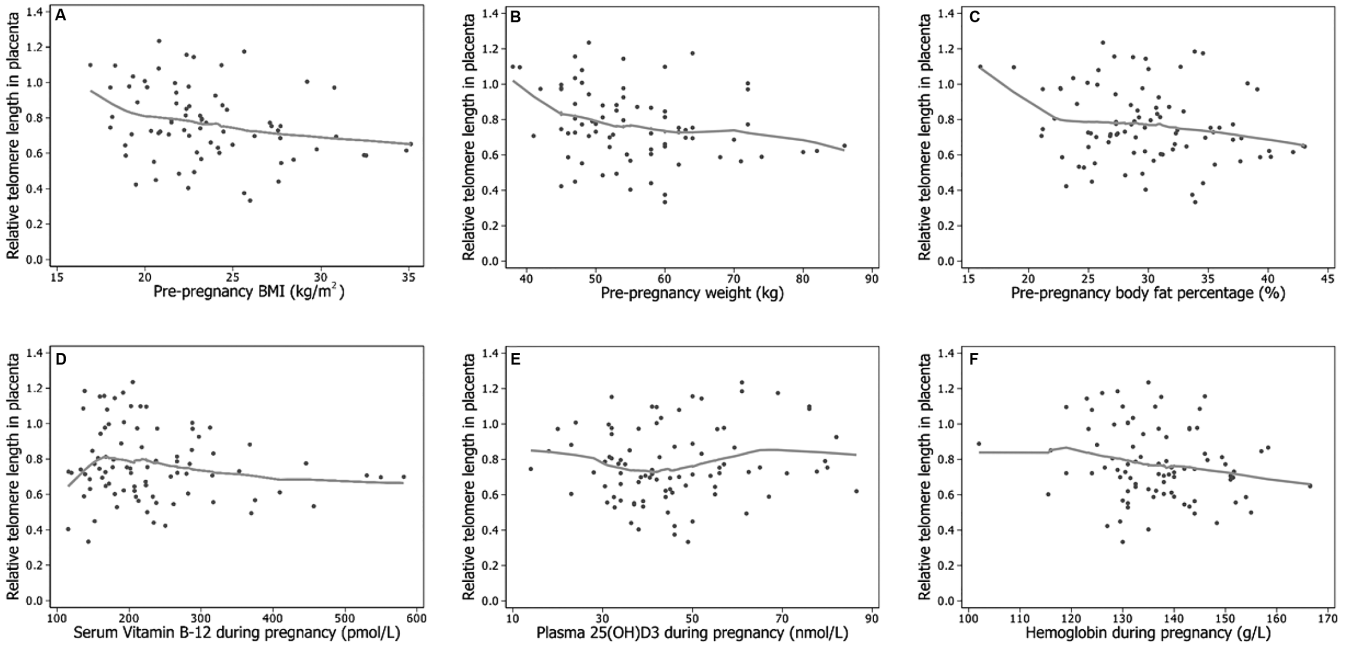

FIGURE 1 Scatterplots with Lowess smoothing curve for relative telomere length in placenta in relation to prepregnancy BMI (A), body weight (B), and body fat mass (C) as well as serum vitamin B-12 (D), plasma 25(OH)D_3 (E), and hemoglobin (F) across pregnancy (mean gestational week 27). 25(OH)D_3, 25-hydroxycholecalciferol.

(**Supplemental Figure 5**), and we performed a spline regression analysis (model 2) with a knot at 22 nmol/L (indicated turning point in the scatterplot). The association with cord blood rTL for women with higher serum folate concentrations became more than twice as strong (β = 0.0069; 95% CI: 0.0009, 0.0128; P = 0.024; n = 84) as in the linear regression. There was no interaction between sex and serum folate (P-interaction = 0.26).

Adjusting for paternal age (range: 16–57 y) instead of maternal age did not change any of the associations between maternal nutritional factors and cord blood rTL (**Supplemental Table 4**). Paternal age was, however, positively associated with cord blood rTL (adjusted for gestational age at birth and birth weight: β = 0.0021; 95% CI: 0.0010, 0.0051; P = 0.18; n = 84), especially for the fathers who were older than ∼30 y, as indicated in the scatterplot (**Supplemental Figure 6**). Applying a spline regression model with a knot at 30 y of age showed a >3 times stronger association above the knot (β = 0.0076; 95% CI: 0.0014, 0.0138; P = 0.017; n = 44). The association with maternal age was weaker (all mothers, β = 0.0015; 95% CI: −0.0021, 0.0052; P = 0.41; n = 92). The few mothers >30 y of age (n = 15) did not allow for a statistical evaluation in this age span.

Discussion

This study indicates that placental rTL is more influenced by variations in maternal nutrition than is cord blood rTL. Maternal prepregnancy body weight, BMI, and BFP were associated with shorter placental rTL, as was serum vitamin B-12. Higher plasma 25(OH)D_3 concentrations, on the other hand, were associated with longer placental rTL. The associations with the vitamins appeared to be nonlinear, with stronger associations at adequate concentrations—that is, > ∼150 pmol/L for vitamin B-12 (34, 35) and >25–30 nmol/L for 25(OH)D_3 (36, 37).

The finding that prepregnancy BMI, a measure of body composition and overall nutrition, was inversely associated with placental rTL is in line with a recent Belgian study, which found that each kg/m² increase in prepregnancy BMI was associated with 0.66% shorter telomeres in the placenta (22). The Belgian women were, on average, 29.1 y old with a mean prepregnancy BMI of 24.1, whereas the women in the present study were almost 5 y younger and leaner (mean prepregnancy BMI: 23.6), especially when considering that they were shorter (∼15 cm shorter than the Belgian women), in agreement with other Andean populations (38). We found that an IQR increase in prepregnancy body weight (48 to 60 kg) was associated with a decrease in placental rTL by ∼0.4 SD, and an IQR increase in prepregnancy BMI (20.8 to 25.6) or BFP (25.8% to 33.2%) was associated with shorter rTL by 0.5 SD. The Belgian study also found a decrease in cord blood telomeres in relation to prepregnancy BMI (22), which was not the case in the present study. A recent meta-analysis concluded that BMI is associated with shorter leukocyte telomeres also in adulthood (39), suggesting that an effect of BMI on rTL may be lifelong.

Telomeres are sensitive to oxidative stress (40, 41). Recent studies have shown that maternal obesity may lead to increased oxidative stress and inflammation in both the placenta and newborn (42–44). This may, at least in part, explain our findings of shortening of the placental rTL with higher maternal BMI. Indeed, we found that particularly BFP in the third trimester was related to shorter rTL, whereas LBM was not. On the other hand, there appeared to be a continuous decrease in placental rTL over the whole range of BMI values (16.9–36.0) in the Andean women, and the Belgian study reported a similar decrease in placental TL for overweight women (BMI: ≥25 to <30) as for obese women (≥30), compared with those with normal weight (22). Thus, there may be other mechanisms in place for a relation between maternal BMI and placental rTL. It is noteworthy that an interplay between oxidative stress, shorter placental TL, epigenetic alterations, and mitochondrial dysfunction has been proposed (5, 45). TL

TABLE 2 Multivariable-adjusted linear regression of nutrition markers during pregnancy with rTL in placenta[1]

Model	*n*	Placenta rTL, B (95% CI)	*P*
Prepregnancy BMI, kg/m^2			
1	89	−0.010 (−0.021, 0.0001)	0.053
2	88	−0.015 (−0.026, −0.005)	0.005
3	81	−0.022 (−0.035, −0.009)	0.001
Prepregnancy body weight, kg			
1	89	−0.004 (−0.009, −0.0002)	0.042
2	89	−0.006 (−0.010, −0.001)	0.011
3	81	−0.007 (−0.012, −0.002)	0.005
Prepregnancy body fat percentage, %			
1	89	−0.008 (−0.016, −0.001)	0.035
2	87	−0.012 (−0.019, −0.004)	0.004
3	81	−0.014 (−0.023, −0.006)	0.002
Height, cm			
1	92	−0.001 (−0.009, 0.007)	0.762
2	91	−0.0001 (−0.008, 0.008)	0.985
3	83	0.0002 (−0.008, 0.007)	0.967
Hemoglobin third trimester, g/L			
1	78	−0.005 (−0.009, −0.001)	0.014
2	75	−0.003 (−0.007, 0.001)	0.126
3	72	−0.002 (−0.006, 0.002)	0.275
Serum folate,[2] nmol/L			
1	85	0.001 (−0.007, 0.009)	0.733
2	82	0.005 (−0.003, 0.013)	0.211
3	81	0.003 (−0.004, 0.011)	0.368
Serum vitamin B-12,[2] nmol/L			
1	85	−0.304 (−0.761, 0.154)	0.190
2	82	−0.497 (−0.950, −0.044)	0.032
3	81	−0.473 (−0.918, −0.028)	0.038
Serum homocysteine,[2] μmol/L			
1	85	−0.004 (−0.039, 0.030)	0.802
2	82	−0.002 (−0.035, 0.032)	0.923
3	81	−0.010 (−0.043, 0.024)	0.566
Plasma 25(OH)D_3,[2] nmol/L			
1	87	0.0024 (−0.0008, 0.0056)	0.135
2	84	0.0039 (0.0009, 0.0069)	0.012
3	81	0.0036 (0.0006, 0.0065)	0.019
Serum selenium,[2] μg/L			
1	88	0.0006 (−0.004, 0.002)	0.696
2	85	0.00002 (−0.003, 0.003)	0.991
3	81	0.0002 (−0.003, 0.003)	0.915
Serum magnesium,[2] mg/L			
1	88	−0.022 (−0.049, 0.006)	0.119
2	85	−0.006 (−0.034, 0.022)	0.673
3	81	0.004 (−0.024, 0.032)	0.769
Serum calcium,[2] mg/L			
1	88	−0.011 (−0.021, −0.002)	0.046
2	85	−0.003 (−0.014, 0.008)	0.565
3	81	−0.001 (−0.012, 0.010)	0.824
Blood zinc,[2] mg/L			
1	87	−0.019 (−0.073, 0.035)	0.484
2	84	−0.019 (−0.070, 0.031)	0.453
3	80	−0.014 (−0.065, 0.036)	0.570
Blood manganese,[2] μg/L			
1	87	0.001 (−0.005, 0.007)	0.709
2	84	0.004 (−0.002, 0.009)	0.212
3	80	0.003 (−0.003, 0.008)	0.333

(*Continued*)

TABLE 2 (*Continued*)

Model	*n*	Placenta rTL, B (95% CI)	*P*
Urine iodine,[2] μg/L, $\log_2$-transformed			
1	88	−0.004 (−0.047, 0.038)	0.846
2	85	−0.013 (−0.052, 0.026)	0.514
3	81	−0.012 (−0.052, 0.028)	0.550

[1] Model 1 is unadjusted. Model 2 is adjusted for mother's age, education, gestational week at delivery, and prepregnancy BMI (if not evaluated variable, or models for weight, height, or body fat mass). The model for plasma 25(OH)D_3 is additionally adjusted for season of sampling. Model 3 is further adjusted for other nutrients, i.e., vitamin B-12, plasma 25(OH)D_3, and season of sampling. rTL, relative telomere length; 25(OH)D_3, 25-hydroxycholecalciferol.
[2] During pregnancy (mean gestational week 27).

may thus be affected by changes in DNA methylation (46), and folate status, which is essential for methylation through one-carbon metabolism, has been found to be positively associated with cord blood TL in US studies (7, 23). The serum folate concentrations in the US mothers (mean: 29.5 ng/mL) (23) were much higher than in the Andean mothers in the present study (mean: 26 nmol/L or 11 ng/mL), probably because all of the US women received supplementation. However, none of the Andean women had a folate concentration <7.5 nmol/L, the lower end of the reference interval (47). Similarly, no woman had a homocysteine concentration >10 μmol/L, the upper reference limit in pregnancy, which also would have indicated folate deficiency (47). Still, when we restricted the analysis to the Andean women with higher serum folate concentrations (>22 nmol/L), the associations between serum folate and cord blood rTL became stronger and statistically significant. An IQR increase in serum folate in this subsample corresponded to an increase in cord blood rTL of 0.35 SD.

Both folate (via formation of 5-methyltetrahydrofolate) and vitamin B-12 (cofactor for methionine synthase) are involved in the conversion of homocysteine to methionine in one-carbon metabolism, and adequate concentrations are important during pregnancy for the development of placenta and fetus (23, 35, 47, 48). Unexpectedly, the present study showed an inverse association of placental rTL with serum vitamin B-12, especially at adequate concentrations, and the association was independent of folate and homocysteine. An IQR increase in serum vitamin B-12 (166–267 pmol/L) was associated with an ~0.2-SD shorter placental rTL. The inverse association was particularly strong in male placentas, in which an IQR increase in serum vitamin B-12 was associated with a 0.5-SD shorter rTL. We found no previous studies on the impact of vitamin B-12 on placental or cord blood TL, but an inverse association between maternal vitamin B-12 status and the expression of placental angiogenesis-related genes has been reported (49). In a study in Colombian children, plasma vitamin B-12 was found to be positively associated with leucocyte TL in girls but not boys (50). Thus, more research in this area is warranted, as vitamin B-12 deficiency is prevalent in populations with low meat intake.

Higher maternal plasma 25(OH)D_3 concentrations, on the other hand, were associated with longer placental rTL. An IQR increase in plasma 25(OH)D_3 (34–55 nmol/L) was associated with a 0.4-SD increase in placental rTL. It should be noted that the plasma 25(OH)D_3 concentrations were generally low. Approximately 58% of the pregnant women in this population had concentrations <50 nmol/L and 19% had concentrations <30 nmol/L (26), indicating deficiency (36, 37). We found no previous data concerning the impact of vitamin D on placental TL, but it is well documented that this vitamin plays a fundamental role in placental development and function (51). An adequate vitamin D status has been associated with lower risk of placental vascular pathologies (52), as well as improved control of placental inflammation (53), which may affect placental telomeres. A recent Korean study (n = 106) reported that maternal 25(OH)D_3 concentrations in pregnancy were positively associated with cord blood TL (25). We found no significant association with cord blood rTL.

Recent NHANES data showed a positive association between dietary selenium intake and TL in adults, suggesting a potential role of selenium in the maintenance of the TL (54). In the present study, we found no difference in placenta or cord blood rTL in relation to the serum selenium concentrations. More than 95% of the pregnant women had serum selenium concentrations >60 μg/L, which is the suggested lower limit of adequate serum concentrations (55).

Paternal age has been associated with longer telomeres in the offspring, related to increasing sperm TL with increasing age (56, 57). In sensitivity analyses we found a stronger association of paternal age than of maternal age with cord blood rTL, particularly for the newborns whose fathers were >30 y. We also found an association of paternal age with placental rTL, but this resembled that of maternal age. Paternal age has previously been found to be positively associated with placenta weight (58); however, this was not the case in the present study.

The clinical significance of the indicated impact of maternal nutritional status on placental rTL is unclear. Premature placental senescence and aging, for which rTL may be a biomarker, have been associated with adverse pregnancy outcomes, such as pre-eclampsia, fetal growth restriction, fetal death, and preterm delivery (59, 60). Little is known, however, about the role of placental rTL for health later in life. A recent study found a significant positive correlation between TL in placenta at birth and TL in buccal cells in early adulthood, although the telomere attrition rate was more pronounced in individuals with longer placental telomeres at birth (10). Evidently, the life course of TL and its implications for health need further studies.

The strengths of the study include the standardized sampling of blood and placenta, measurement of multiple maternal nutritional factors, and collection of information on other potential influential factors for TL. Essentially all of the women in the study were nonsmokers with only occasional alcohol consumption, and the study area has minimal industrial or traffic pollution, factors that may affect fetal rTL. Limitations of the study include the small number of placenta and cord blood samples in the cohort and that we were not able to collect fasting blood and urine samples. Also, we measured cord blood leukocyte telomeres without adjusting for variations in leukocyte types or for placenta cell type. Further analysis of telomerase activity and markers of oxidative stress could have shed some light on the potential mechanisms.

TABLE 3 Multivariable-adjusted linear regression of nutrition markers during pregnancy and rTL cord blood[1]

Model	*n*	Cord blood rTL, B (95% CI)	*P*
Prepregnancy BMI, kg/m^2			
1	88	−0.002 (−0.007, 0.004)	0.604
2	88	−0.002 (−0.008, 0.004)	0.511
Prepregnancy body weight, kg			
1	90	−0.001 (−0.003, 0.002)	0.489
2	89	−0.001 (−0.004, 0.002)	0.404
Prepregnancy body fat percentage, %			
1	87	0.0002 (−0.005, 0.004)	0.913
2	87	−0.001 (−0.006, 0.004)	0.682
Height, cm			
1	91	−0.001 (−0.006, 0.003)	0.558
2	91	−0.001 (−0.005, 0.004)	0.711
Hemoglobin third trimester, g/L			
1	78	−0.001 (−0.003, 0.001)	0.427
2	78	−0.001 (−0.004, 0.001)	0.207
Serum folate,[2] nmol/L			
1	84	0.003 (−0.001, 0.007)	0.195
2	84	0.003 (−0.002, 0.007)	0.193
Serum vitamin B-12,[2] nmol/L			
1	84	−0.069 (−0.324, 0.186)	0.591
2	84	−0.095 (−0.350, 0.159)	0.458
Serum homocysteine,[2] μmol/L			
1	84	−0.009 (−0.028, 0.010)	0.336
2	84	−0.008 (−0.026, 0.011)	0.416
Plasma 25(OH)D_3,[2] nmol/L			
1	86	0.001 (−0.001, 0.003)	0.255
2	86	0.001 (−0.001, 0.003)	0.406
Serum selenium,[2] μg/L			
1	87	0.0008 (−0.001, 0.002)	0.376
2	87	0.0006 (−0.001, 0.002)	0.488
Serum magnesium,[2] mg/L			
1	87	−0.004 (−0.019, 0.011)	0.613
2	87	−0.006 (−0.021, 0.008)	0.406
Serum calcium,[2] mg/L			
1	87	−0.002 (−0.008, 0.004)	0.571
2	87	−0.003 (−0.009, 0.004)	0.399
Blood zinc,[2] mg/L			
1	86	−0.014 (−0.043, 0.015)	0.343
2	86	−0.013 (−0.043, 0.016)	0.373
Blood manganese,[2] μg/L			
1	86	0.0002 (−0.0033, 0.0029)	0.913
2	85	0.0001 (−0.0030, 0.0032)	0.939
Urine iodine,[2] μg/L, $\log_2$-transformed			
1	87	−0.002 (−0.025, 0.021)	0.877
2	87	−0.002 (−0.021, 0.026)	0.853

[1] Model 1 is unadjusted. Model 2 is adjusted for mother´s age, gestational week at birth, and birth weight. The model for plasma 25(OH)D_3 is additionally adjusted for season of sampling. rTL, relative telomere length; 25(OH)D_3, 25-hydroxycholecalciferol.
[2] During pregnancy (mean gestational week 27).

In conclusion, this study indicates that maternal nutritional status before and during pregnancy may predict TL in the placenta. In particular, maternal BMI, BFP, and vitamin B-12 status were associated with shorter placental rTL, whereas vitamin D was predictive of longer placental telomeres. The results contribute to a better understanding of the biological mechanisms underlying effects of nutritional variations in pregnancy. However, more research is needed, not the least concerning potential effects of altered placental telomeres on child health. Such effects may have long-term public health impact, as the TL at birth is hypothesized to predict the TL for life.

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