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The Effect of Vitamin B_{12} Supplementation on Leukocyte Telomere Length in Mildly Stunted Nepalese Children: A Secondary Outcome of a Randomized Controlled Trial

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A B S T R A C T

Background: Vitamin B_{12} is essential for deoxyribonucleic acid synthesis and genome stability. A deficiency of vitamin B_{12} is associated with telomere shortening, genomic aging, and increased risk of chronic disease and mortality.
Objectives: The study aims to determine the effect of vitamin B_{12} supplementation on leukocyte telomere length (LTL) in infants at risk of vitamin B_{12} deficiency.
Methods: The study was a predefined secondary analysis of a randomized controlled trial enrolling 600 Nepalese infants aged 6 –11 mo, who were supplemented with 2 μg (2–3 recommended daily allowances) vitamin B_{12} or placebo daily for 1 y. At the end of the study, LTL was measured in 497 participants. Mean LTL was compared between the treatment arms in the full sample and predefined subgroups based on markers of vitamin B_{12} status, hemoglobin, sex, and growth indices.
Results: LTL at end-study did not differ between the vitamin B_{12} and placebo arm with a standardized mean difference (95% confidence interval) of 0.04 (–0.14, 0.21). There was no effect of vitamin B_{12} on LTL in any of the subgroups.
Conclusions: Providing daily vitamin B_{12} for 1 y during infancy in a population at risk of vitamin B_{12} deficiency does not affect LTL. This trial was registered at clinicaltrials.gov as NCT02272842.

Keywords: leukocyte telomere length, vitamin B_{12} supplementation, children, Nepal

Introduction

Telomeres cap the end of each DNA strand to protect the chromosomes [1,2] and play a vital role in cellular survival by maintaining chromosomal stability during cell division. Telomere length (TL), which shortens naturally during each cell division in the DNA replication cycle, is associated with biological age and is hence considered a biomarker of the aging process [3]. Some studies in adults observed TL shortening in diseases such as cancer, diabetes, chronic obstructive pulmonary disease, and obesity [4–6], as well as environmental and lifestyle-related factors such as socioeconomic status, smoking, dietary antioxidant intake, and multivitamin supplementation [7–11].

Several studies have linked micronutrient status with TL [1, 12,13]. The B-vitamins B_6, B_9 (folate), and B_{12} (cobalamin), all of which are involved in the one-carbon metabolism (OCM) and related to DNA methylation and synthesis, have been linked to TL [14]. Results from observational studies on OCM-related

Abbreviations: Ct, cycle threshold; Hb, hemoglobin; LAZ, length-for-age z-score; LTL, leukocyte telomere length; MMA, methylmalonic acid; OCM, one-carbon metabolism; RCT, randomized controlled trial; tHcy, total homocysteine; TL, telomere length; $3cB_{12}$, combined indicators of vitamin B_{12}.

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biomarkers and TL have shown conflicting results [15–17]. However, studies undertaken in early life have found positive associations between vitamin B_{12} and TL [18,19]. If poor vitamin B_{12} status accelerates telomere shortening, measures should be sought to improve vitamin B_{12} status in early life. A causal effect of this vitamin on TL should be established in randomized controlled trials (RCTs).

In Bhaktapur, Nepal, we have observed poor vitamin B_{12} status in young children, indicated by low plasma cobalamin, elevated total homocysteine (tHcy), and methylmalonic acid (MMA) concentrations [20,21]. In a recently conducted RCT, we found a substantial biochemical response in these functional markers following 1 y of vitamin B_{12} supplementation [22].

The most significant telomere attrition happens within the first year of life, continues up to age 4 y, and is followed by a slower attrition rate throughout adulthood [23]. The primary objective of the current study was to measure the effect of vitamin B_{12} supplementation on leukocyte TL (LTL) among young children and explore the extent to which selected baseline features modify the effect of vitamin B_{12} on LTL [22].

Methods

Study design and participants

The study was a predefined secondary analysis of a randomized, double-blind, placebo-controlled trial conducted in children residing in Nepal's Bhaktapur municipality and surrounding peri-urban communities. Bhaktapur municipality is 1 of the most densely populated (11,340 people/km^2) municipalities in Nepal [24]. Most people in the community are from the Newar ethnic group. Although it is a predominantly nonvegetarian population, their intake of animal-based foods is low [25]. Anemia, malnutrition, vitamin $B_{12,}$ and zinc deficiency are common, whereas folate deficiency is rare [21,26].

Enrolment and randomization

A total of 600 children aged 6–11 mo with a length-for-age *z*-score (LAZ) <–1, who planned to reside in the community for the next 12 mo and whose parents provided written consent were enrolled from April 2015 to February 2017. Children were excluded if they had an acute or chronic illness, severe malnutrition, severe anemia [Hemoglobin (Hb) <70 g/L], or taking multivitamin supplements containing vitamin B_{12} or required treatment with vitamin B_{12}.

Field workers identified eligible children from immunization/vaccination clinics or through home visits, and the infants were enrolled by a study supervisor or a physician at the field office. We randomly assigned the infants in a 1:1 ratio in blocks of 8 using a computer-generated randomization list to either vitamin B12 supplements or placebo. Participants were linked to the study arm through the identification number printed on the supplement labels. The list that linked this identification number to the randomization code was kept with the producers of the supplements and the independent scientists who generated it. None of the investigators had access to this list until the data collection and data cleaning for the primary outcomes were completed. Details on the study protocol and the primary outcomes have been published elsewhere [20,22].

Intervention

All children received a daily oral placebo or supplements for 12 mo with 2 μg vitamin B_{12} [corresponding to ~2–3 recommended daily allowances (RDA)] [22]. The vitamin B_{12} supplements and placebo were produced specifically for the trial and were identical in taste and appearance. Both the intervention and placebo supplements were packed in metalized foil sachets containing 20 g of a lipid-based nutrient paste with 108 kcal and an equal quantity of other multivitamins and minerals (vitamins B_1, B_2, B_3, B_5, B_6, calcium, magnesium, iron, zinc, copper, iodine, selenium, phosphorus, and potassium) at approximately 1 RDA which is the daily requirement dose for children [22]. The nutrient pastes were produced by GC Rieber-Compact (http://www.gcrieber-compact.com).

Compliance

The first supplement was given at the field site clinic under the observation of study personnel. During weekly visits to the homes, trained field workers asked the mothers about the intake of the paste during the last 7 d. The fieldworkers recorded the amount of paste given to the children in detail and counted the total number of empty paste sachets to verify the reported compliance. Among the children who completed the 1-y supplementation, >94% of the prescribed doses were taken in both arms [22].

Data collection

At enrolment, trained field workers collected information on the family's household characteristics and socioeconomic situation, feeding practices, as well as the child's morbidity, hospitalization, and birth history. Children's weight and length were measured by trained field workers at the study clinic according to standard guidelines using portable electronic scales and infantometers (model 417; Seca).

Blood sampling and biochemical analyses

Blood samples from the participating children were collected at the study clinic at enrolment before the start and at the end of supplementation at age 18–23 mo. The 3–4 mL of blood was collected from 1 of the cubital veins into polypropylene tubes containing EDTA (Sarstedt). Hb concentration was analyzed immediately following blood sampling with Hemocue, which was calibrated as per the guidelines defined by the manufacturer. The blood was centrifuged for 10 min at 2000–2500 g within 10 min of venipuncture. Plasma was separated and transferred into polypropylene vials (Eppendorf). These vials were kept at −196°C in liquid nitrogen at the field site clinic before being transferred to Walter/Reed Armed Forces Research Institute of Medical Science (AFRIMS) Research Unit Nepal (WARUN), where they were stored at –80°C until transferred to Norway on dry ice. All samples were kept frozen until thawing for analysis. All biochemical analyses related to vitamin B_{12} metabolism were performed at Bevital AS, Bergen, Norway (www.bevital.no). Plasma cobalamin was analyzed using a microbiological assay based on colistin sulfate-resistant strains of Lactobacillus leichmannii, and folate was analyzed using a chloramphenicol-resistant strain of Lactobacillus casei [27,28]. Plasma tHcy and MMA were analyzed by gas

chromatography-tandem mass spectrometry-based on methyl chloroformate derivatization [29].

LTL assay

LTL was measured from the blood samples collected at the end of supplementation when children were 18–23 mo. Because of limited resources, we could not analyze LTL at baseline. Five nanograms of genomic DNA were dried down in each 384-well plate and resuspended in 10μL of either the telomere or 36B4 polymerase chain reaction mixture and stored at 4°C for $\leq$6 h. The telomere reaction mixture consisted of 1x Thermo Fisher PowerUP SYBR Master Mix, 2.0 mM of dithiothreitol (DTT), 270 nM of Tel-1b primer, and 900 nM of Tel-2b primer. The reaction proceeded for 1 cycle, hold at 50°C for 2 min and at 95°C for 2 min, followed by 35 cycles at 95°C for 15 s and 54°C for 2 min. The 36B4 reaction consisted of 1x Thermo Fisher PowerUP SYBR Master Mix, 300 nM of 36B4U primer, and 500 nM of 36B4D primer. The 36B4 reaction proceeded for 1 cycle hold at 50°C for 2 min and at 95°C for 2 min, followed by 40 cycles at 95°C for 15 s and 58°C for 1 min and 10 s. All samples for both the telomere and single-copy gene (36B4) reactions were performed in triplicate on different plates. Each 384-well plate also contained a 6-point standard curve from 0.625 ng to 20 ng using pooled buffy-coat-derived genomic DNA.

The standard curve assessed and compensated for inter-plate variations in polymerase chain reaction efficiency. The slopes of the standard curve for both the telomere and 36B4 reactions were –3.33 $\pm$ 0.33, and the linear correlation coefficient (R^2) values for both reactions were over 0.99. The telomeric DNA with the single copy of beta-globin gene (T/S ratio) (-dCt) for each sample was calculated by subtracting the mean 36B4 threshold cycle (Ct) value from the mean telomere Ct value. The relative T/S ratio (-ddCt) was determined by subtracting the T/S ratio value of the 5 ng standard curve point from the T/S ratio of each unknown sample. Quality control samples were interspersed throughout the test samples in order to assess inter-plate and intra-plate variability of Ct values. A combined inter- and intra-assay coefficient of variation calculated from the relative T/S ratio (-ddCt) of quality control samples is 8.5%. The LTL analysis was done in the Research Laboratory at Harvard School of Public Health, Boston, USA.

Definitions

The cut-off value to define anemia, adjusted for the local altitude of 1400 m as per the WHO/Centers for Disease Control and Prevention guidelines, was 11.3 g/dL [30]. Low cobalamin, low folate, high MMA, and high tHcy were defined as plasma values <150 pmol/L [31], <10 nmol/L [31], >0.26 μmol/L, and >10 μmol/L, respectively. The combined indicator for cobalamin status ($3cB_{12}$) was calculated from cobalamin, MMA, and tHcy, as suggested by Fedosov et al. [32]. A $3cB_{12}$ score <-0.5 was used to indicate low vitamin B_{12} status. Underweight, stunting, and wasting were defined as weight-for-age, length-for-age, and weight-for-length below -2 z-scores according to the WHO Child Growth Standard [33]. We used the guidelines of Labbok and Krasovec [34] to define exclusive breastfeeding. According to this definition, a child is considered exclusively breastfed if he/she was given only the mother's milk except medicine since birth. Information on whether the child was exclusively breastfed up to the age of 6 mo was collected at the time of enrolment. Preterm was defined if the child was born <37 weeks of gestation, and low birth weight was defined if the child was born <2500 g.

Sample size

The sample size calculation was based on the primary outcomes of the study, which were growth and neurodevelopment scores. With the given sample size of 497 children and LTL as outcomes, we had 80% and 92% power to detect standardized effect sizes of 0.25 and 0.30, respectively.

Ethics

The study has obtained approval from the Nepal Health Research Council (NHRC, #233/2014) in Nepal and the

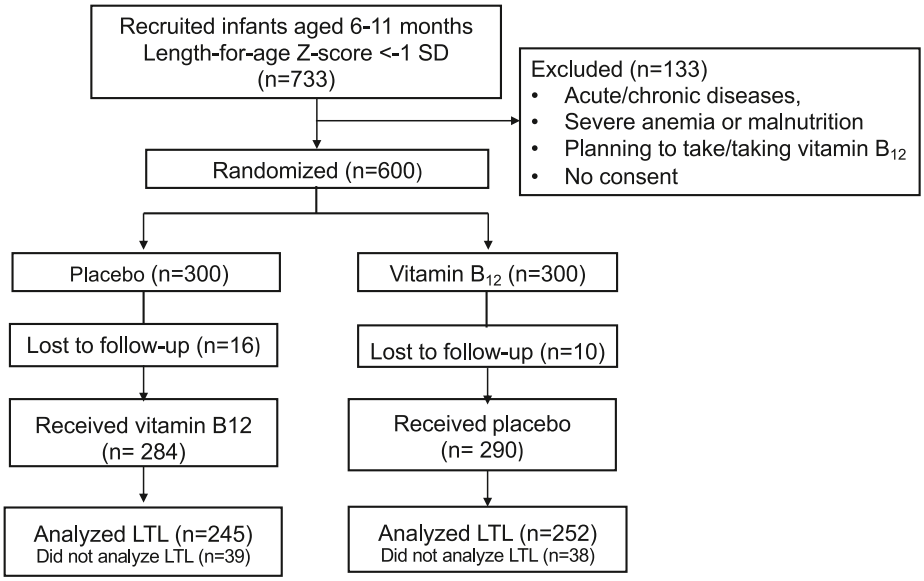

FIGURE 1. Study flow chart of a randomized placebo-controlled trial of daily vitamin B_{12} supplementation on leukocyte telomere length in Nepalese infants. LTL, leukocyte telomere length; SD, standard deviation.

Regional Committee for Medical and Health Research Ethics (REC #2014/1528) in Norway. Written informed consent from 1 of the parents was obtained; for those who were illiterate, signed consent was provided by relatives or witnessed on the parent's behalf. The study was implemented in accordance with the Helsinki Declaration.

Statistical analysis

All data were checked manually by the supervisors for consistency and double-entered in Microsoft Access and a digital data capture system (https://www.zerionsoftware.com/iformbuilder). Statistical analyses were undertaken using Stata, version 18 (StataCorp LLC). Data were presented as numbers and proportions (percentage) for all categorical variables or mean and SD for continuous baseline variables, calculated for both study arms separately. For the analyses, we used both relative LTL and standardized LTL measurements. We compared the mean LTL between the vitamin B_{12} and the placebo arm in linear regression and in an intent-to-treat approach. We present the effect of vitamin B_{12} supplementation from crude and confounder-adjusted linear regression models. In the adjusted models, we adjusted for sociodemographic status (maternal age, rented or owned home, joint or nuclear family, and maternal education), whether firewood was the main source of cocking fuel, child age, and sex. The rationale for selecting these variables was that they were potential or observed predictors for LTL. We depicted the distribution of LTL in the vitamin B_{12} and placebo arm using Epanechnikov kernel density plots. We used confounder-adjusted linear regression models to estimate the association between LTL and the intervention in predefined subgroups. We used the same subgroups as in the manuscript reporting the main outcomes of the trial and adjusted for the same variables as in the overall analyses. These subgroups are based on the plasma concentration of biomarkers reflecting vitamin B_{12} status (cobalamin, tHcy, MMA, and $3cB_{12}$), Hb, birth weight, length, weight, exclusive breastfeeding status at 6 mo, and sex (sex was not included as a subgrouping variable in the original manuscript). The subgroup-specific effects are presented in forest plots.

Results

Among the 578 children who completed supplementation, LTL was analyzed in the first 497 enrolled children. Detailed information concerning recruitment and follow-up is shown in Figure 1.

General characteristics

Demographics, breastfeeding, and perinatal characteristics for the intervention, placebo arm, and the 103 participants who were not included in the LTL analyses were similar (Tables 1 and 2). The mean age of the children at enrolment was 8 mo (SD 1.8). One in every 5 children was born with low birth weight, and ~10% were born preterm. Approximately 10% of the children were

TABLE 1
Baseline characteristics of the 600 enrolled infants by study arm and if included in the current analysis

Characteristics	Vitamin B_{12}	Placebo	Not included[2]
	$N = 252$	$N = 245$	$N = 103$
Infant characteristics			
Age of child (mo)	7.9 (1.8)	8.0 (1.8)	8.4 (1.8)
Sex, male	130 (51.6%)	126 (51.4%)	53 (51.5%)
Cesarean section	82 (32.5%)	83 (34.0%)	18 (17.5%)
Birth weight in grams	2809 (503)	2781 (507)	2752 (462)
Low birth weight[1] (<2500 g)	47 (18.7%)	46 (18.8%)	22 (21.4%)
Preterm birth (<37 weeks of gestation)	23 (9.1%)	30 (12.2%)	9 (8.7%)
Hospitalized after the neonatal period	7 (2.8%)	15 (6.1%)	4 (3.9%)
Maternal and paternal characteristics			
Age of mother (y)	27.7 (4.6)	27.4 (4.7)	26.2 (4.7)
Age of father (y)	30.7 (5.1)	29.8 (5.6)	30.8 (9.6)
Illiterate mothers	24 (9.5%)	18 (7.3%)	6 (5.8%)
No occupation of mother	30 (11.9%)	29 (11.8%)	14 (13.6%)
No ownership of the vehicle	146 (57.9%)	137 (55.9%)	65 (63.1%)
Family ownership of land	123 (48.8%)	119 (48.6%)	40 (38.8%)
Receiving remittance from abroad	20 (7.9%)	28 (11.4%)	9 (8.7%)
Biomass is the main source of cooking fuel	45 (17.9%)	48 (19.6%)	14 (13.6%)
Child anthropometry			
Weight for age *z*-score (WAZ)	–0.2 (1.0)	–0.2 (1.0)	–0.3 (0.9)
LAZ	–1.2 (0.8)	–1.3 (0.9)	–1.4 (0.8)
Weight-for-length *z*-score (WLZ)	–1.8 (0.6)	–1.8 (0.6)	–1.9 (0.6)
Wasting (WLZ <–2)	6 (2.4%)	10 (4.1%)	3 (2.9%)
Stunting (LAZ <–2)	79 (31.3%)	76 (31.0%)	39 (37.9%)
Underweight (WAZ <–2)	39 (15.5%)	49 (20.0%)	24 (23.3%)
Breastfeeding status:			
Breastfeeding at the time of interview	246 (97.6%)	237 (96.7%)	103 (100.0%)
Exclusive breastfeeding ≥6 mo	30 (11.9%)	28 (11.4%)	12 (11.7%)
Exclusive breastfeeding ≥3 mo	117 (46.4%)	117 (47.8%)	46 (44.7%)
Started complementary feeding ≤1 mo	45 (17.9%)	40 (16.3%)	19 (18.4%)

LAZ , Length-for-age z-score
[1] Among 480 newborns, where birth records were available, *n*, number.
[2] Leucocyte telomere length not measured.

TABLE 2
Baseline hemoglobin and plasma cobalamin status of the 600 enrolled infants by study arm and if included in the current analysis

	Vitamin B_{12}	Placebo	Not included[3]
	$N = 252$	$N = 245$	$N = 103$
Hemoglobin status:			
Mean hemoglobin, g/dL	10.5 (10.0, 11.2)	10.5 (9.9, 11.3)	10.5 (10.0, 11.2)
Hemoglobin <11.0 g/dL	167 (66.3%)	152 (62.0%)	68 (66.0%)
Hemoglobin <11.3 g/dL[1]	191 (75.8%)	179 (73.1%)	79 (76.7%)
Vitamin B_{12}-related biomarkers:			
Cobalamin, pmol/L	233.0 (173.7, 319.6)	260.5 (192.1, 356.3)	248.0 (196.7, 379.4)
Cobalamin <150 pmol/L	44 (17.5%)	29 (11.9%)	16 (15.7%)
Folate, μmol/L	61.5 (46.9, 79.2)	57.0 (42.8, 79.9)	67.7 (52.8, 82.9)
Folate <10 μmol/L	0	0	0
Methylmalonic acid, μmol/	0.4 (0.2, 0.8)	0.4 (0.3, 0.8)	0.5 (0.3, 0.8)
Methylmalonic acid >0.26 μmol/L	184 (73.0%)	182 (74.3%)	82 (80.4%)
Total homocysteine,	10.1 (7.8, 13.6)	10.4 (7.9, 14.0)	11.1 (8.1, 15.5)
Total homocysteine >10 μmol/L	127 (50.4%)	129 (52.7%)	63 (61.8%)
$3cB_{12}$[2]	–0.7 (–1.3, –0.2)	–0.7 (–1.2, –0.1)	–0.7 (–1.3, –0.2)
$3cB_{12}$ <–0.5	154 (61.1%)	138 (56.6%)	65 (63.7%)

MMA, methylmalonic acid; tHcy, total homocysteine; 3cB12, combined indicators of vitamin B_{12}.
[1] Adjusted for the altitude of Bhaktapur Nepal.
[2] 3cB12 (combined indicators of 3 biomarkers: vitamin B_{12}, tHcy, and MMA) was calculated by the log of cobalamin over the product of tHcy and MMA concentrations, minus an age factor {log10[(B_{12})/(MMA·tHcy)] – (age factor)}.
[3] Leucocyte telomere length not measured.

exclusively breastfed for 6 mo or more. Early introduction of complementary feedings was common, with 17% of the children being introduced to complementary feeding within 1 mo of birth. One-third of the children were stunted at enrolment (LAZ <–2 *z*-score). Almost half of the participants lived in joint families and resided in rented houses. At baseline, 72% of the children were anemic (Hb <11.3 g/dL), and 14% of the children were vitamin B_{12} deficient (plasma cobalamin <150 pmol/L), whereas none were folate deficient. About two-thirds of the children had elevated MMA (>0.26 μmol/L), >50% had elevated tHcy (>10 μmol/L), and >40% of the children had low 3cB12 (<–0.5) (Table 2).

LTL

The distribution of LTL among the vitamin B_{12} and placebo arms is shown in Figure 2. At the end of supplementation, the relative mean (SD) of LTL was 1.02 (0.20) units in the vitamin B_{12} arm and 1.03 (0.18) units in the placebo arm with a mean difference (95% confidence interval) of 0.007 (–0.026 to 0.041) and standardized mean difference of 0.04 (–0.14, 0.21) (Table 3). The observed effect was not altered when adjusting for potential confounders. The effect of vitamin B_{12} supplementation on LTL in predefined subgroups is shown in Figure 3. There were no differences in LTL between the study arms in any of these subgroups.

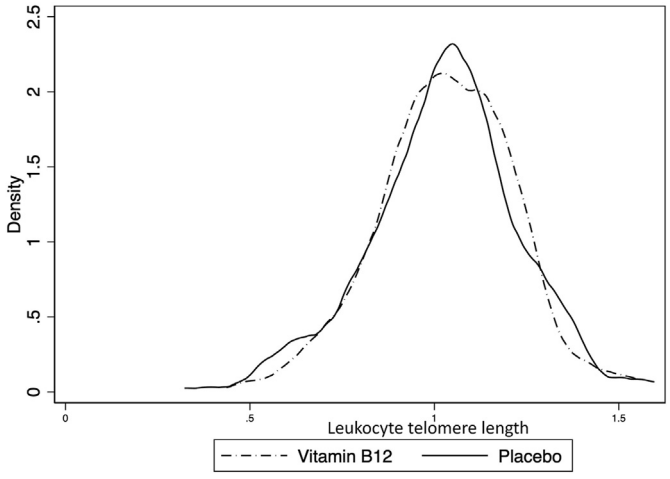

FIGURE 2. Distribution of the leukocyte telomere length by study arm in a randomized placebo-controlled trial in Nepalese children at age 18–23 mo old.

Discussion

This RCT in young Nepalese children examined the effect of vitamin B_{12} supplementation on LTL. Despite very good compliance to the vitamin B_{12} supplementation reflected both in supplementation consumption (>94% of the scheduled doses were taken) as well as improved vitamin B_{12} status, we did not find any effect of the vitamin B_{12} supplementation on LTL in the full sample or predefined subgroups. There is substantial intra-individual variability in LTL in early life [35]. If we had measured LTL both at baseline and at the end of the study, we could have taken this variability into account. This potential noise could have affected the precision of the effect estimates but would not result in biased estimates. In other words, it is possible that we could have identified small differences if LTL also had been measured at baseline. Furthermore, not having baseline LTL measurements also increases risk of confounding bias. However, there were 250 individuals in each of the study groups, which minimizes risk of such bias.

Vitamin B_{12} is required for essential metabolic functions and acts as a coenzyme in converting tHcy to methionine [14,18]. Methionine is the major methyl group donor used mainly in the methylation of DNA and RNA [36]. Low vitamin B_{12} status leads to increased tHcy and elevated oxidative stress, which is 1 proposed mechanism for shortening of LTL and impaired maintenance of genome integrity [37–40]. To the best of our knowledge, this is the first study to investigate the effect of

TABLE 3
The leukocyte telomere length by study arms among 18–23 mo Nepalese children

	Vitamin B_{12} $N = 245$ Mean ± SD	Placebo $N = 252$ Mean ± SD	Mean difference (95% CI)
T/S ratio	1.03 ± 0.18	1.02 ± 0.20	0.007 (–0.026, 0.041)
T/S ratio *z*-score	0.02 ± 0.95	–0.02 ±1.1	0.04 (–0.14, 0.21)
Adjusted T/S ratio *z*-score, adjusted[1]			0.03 (–0.14, 0.20)

Estimates are from linear regression models, [1]adjusted for sex, baseline vitamin B12 status, socioeconomic status, WAZ, whether biomass was the main cooking fuel, and age.
CI, confidence interval; WAZ, weight for age z-score; T/S, telomeric DNA (T) with single copy (S) beta-globin gene.

vitamin B_{12} supplementation on LTL in children. The available literature investigating biomarkers in the OCM pathway, such as folate, vitamin B_{12}, and tHcy in relation to LTL, are primarily based on observational studies in adults [41]. One-year supplementation with vitamin B_{12} combined with vitamin B_6 and folate resulted in longer telomeres in a pilot study among elderly people [41]. Moreover, a cross-sectional study showed a positive association between LTL and vitamin B_{12} status in 5–12-y-old Colombian school girls [19], and another study showed that poor maternal vitamin B_{12} was associated with shorter LTL in their newborns [18]. On the contrary, 3 studies among adults in the United States found no relation between the status or dietary intake of vitamin B_{12} folate with LTL [17,38,39].

We analyzed LTL at 18–23 mo of age, which is a period of substantial telomere attrition and a great time to study the effect of interventions on LTL [42]. It is possible that by including a different population and in a different study design, we could have observed different results. Although the current study included children with LAZ <–1 to target vitamin B_{12} deficient children, only about half had biochemical signs of deficiency (3cB12 <–0.5). Moreover, a dose of 2–3 RDA may not be sufficient to replenish the vitamin B_{12} stores in deficient children. By including children with known signs of deficiency and/or providing a larger dose, we could have observed different results.

Our study has several strengths, which include intensive and regular follow-up, high compliance with the intervention supplement, and very few dropouts. The collection and transportation of blood and the measurement of LTL were carried out according to standard protocols and quality control. There were

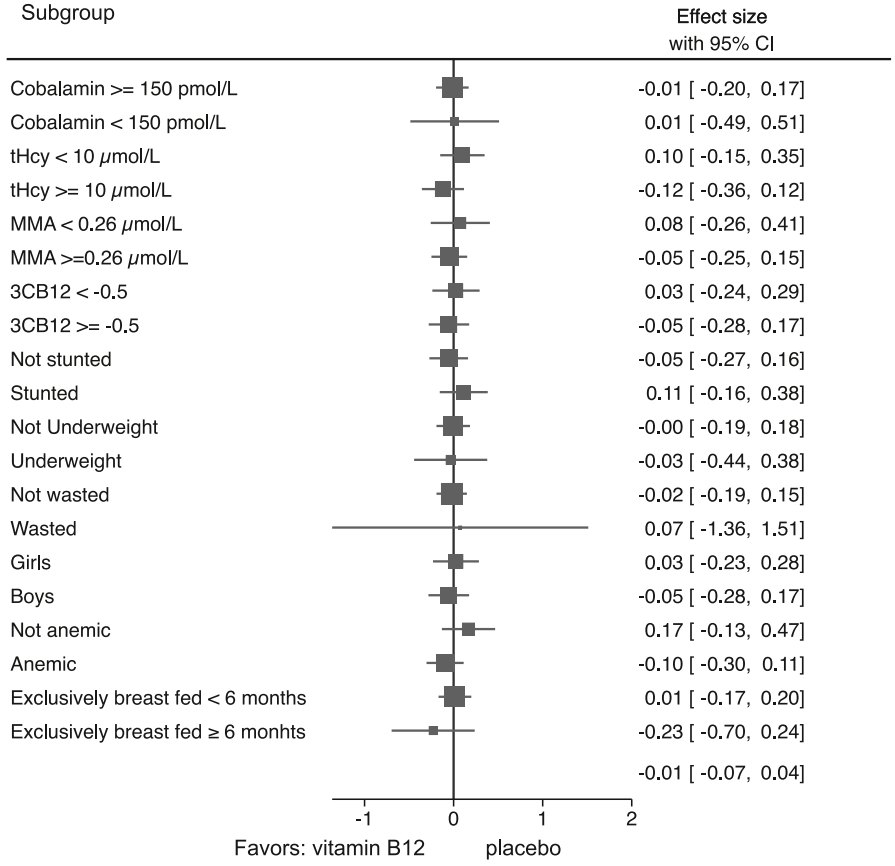

FIGURE 3. Effect of vitamin B_{12} supplementation on leukocyte telomere length in predefined subgroups in a randomized placebo-controlled trial in Nepalese children at age 18–23 mo old. A point estimate to the left of the vertical line indicates a beneficial effect of vitamin B_{12}. 3cB12is a combined vitamin B_{12} status indicator [32]. 3cB12 is a function of the plasma concentration of cobalamin, tHcy, and MMA. CI, confidence interval; MMA, methylmalonic acid; tHcy, total homocysteine; 3cB12, combined indicators of vitamin B12.

some limitations of our study, such as not being able to measure LTL at baseline. Because of the enrolment restriction of infants with LAZ <−1 SD, our findings may not be readily generalized to other infant populations. The supplemented paste given to both study arms also contained many other micronutrients at the levels of ~1 RDA, which potentially could alter the effect of the vitamin B_{12} intervention.

In conclusion, daily supplementation with ~2–3 RDAs of vitamin B_{12} during infancy for 1 y among mildly stunted Nepalese infants did not alter their LTL.

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Author contributions

The authors' responsibilities were as follows– MU, RKC, IK, and TAS: designed the randomized controlled trial (RCT); MU, RKC, MS, and SR: implemented the RCT; PMU, AM, LVN, DC-P, and IDV: analyzed blood samples; MU, IK, TAS, RKC, and CS: planned the analysis and conducted the statistical analysis; MU, IK, TAS, CS, SB, and RKC: wrote the first draft of the paper; MU and TAS had primary responsibility for final content, and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data is available on request. In order to meet ethical requirements for the use of confidential patient data, requests must be approved by the Nepal Health Research Council and the Regional Committee for Medical and Health Research Ethics in Norway. Requests for data should be sent to the authors by contacting the Department of Pediatrics, Institute of Medicine, Tribhuvan University (chrp2015@gmail.com), or by contacting the Department of Global Health and Primary Care at the University of Bergen (post@igs.uib.no).

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Further reading

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