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Original article

Maternal intake of milk and milk proteins is positively associated with birth weight: A prospective observational cohort study☆

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SUMMARY

Background: A striking number of low birth weight (LBW) Indian babies are born annually. Previous studies have confirmed the positive association between milk intake and birth weight. However, the relations between protein and vitamin B_{12} from milk and birth weight have not been systematically explored.
Aims: We examined the relations between birth weight and maternal intake of milk, protein from milk and vitamin B_{12} from milk.
Methods: This prospective, observational cohort study was conducted in an urban South Indian hospital. The dietary intakes of milk and milk products were assessed using validated food frequency questionnaire and at delivery birth outcomes were measured. The relations between milk products, milk protein, and vitamin B_{12} from milk with birth weight and gestational weight gain were assessed in 2036 births with first trimester dietary and delivery data.
Results: Median consumption of milk products in the first trimester was 310 $g \cdot day^{-1}$ and average birth weight was 2876 g. Birth weight was positively associated with intake of milk products and of % protein from milk products (%milk protein) in the first trimester [$\beta = 86.8$, 95% confidence interval (CI): 29.1, 144.6; $\beta = 63.1$, 95% CI: 10.8, 115.5; $P < 0.001$ for both]. Intake of milk products and of %milk protein in the third trimester was positively associated with gestational weight gain (GWG) between the second and third trimester (One-way ANOVA, $P < 0.001$ and $= 0.001$, respectively). Neither birth weight nor GWG were associated with %vitamin B_{12} from milk products.
Conclusions: These findings indicate that intake of milk products in the first trimester and especially, protein from milk products is positively associated with birth weight in this South Asian Indian population.

1. Introduction

India has an unacceptable rate of low birth weight (<2500 g) babies born every year [1]. LBW and pre-term babies accounted for 14% of all neonatal deaths in 2005 while neonatal deaths, in turn, were responsible for 43% of deaths under five years of age [2]. Among the modifiable factors affecting birth weight, maternal nutrition has time and again been shown to be intimately related to birth outcomes [3,4].

Though findings from human studies are equivocal in terms of associations between maternal dietary protein intake and birth outcomes, milk consumption has proven important to birth weight

Abbreviations: SGA, small for gestational age; AGA, appropriate for gestational age; LBW, low birth weight.

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[5–7]. Studies in Denmark and Rotterdam have shown that antenatal milk intake is positively related to birth weight—although the mechanisms by which milk intake increased birth weight could be different. The study of the Danish National Birth Cohort—including 50117 mothers and their infants—compared mothers who consumed six or more glasses of milk per day to those who consumed none. It was found that mothers who drank six or more glasses had an increased risk of having a large for gestational age (LGA) baby, but a lower risk of having a small for gestational age (SGA) baby; in fact, the risk of an SGA baby was almost half [8]. The Generation R study in Rotterdam found that consuming more than three glasses of milk per day was found to be associated with an 88 g increase in birth weight in the third trimester as opposed to one glass or none at all [9]. Earlier studies have demonstrated the significant positive association between protein intake and birth weight [10]. Balanced energy and protein intake during pregnancy has been reported to be positively associated with birth weight in 13 controlled trials of a total of 4665 women [11]. Vitamin B_{12} appears to have an important role in the intrauterine development of the fetus—particularly in neurological and other development of the growing baby [12]. In South Indian women, who are predominantly vegetarian, low maternal B_{12} intakes and levels in all three trimesters are associated with a higher risk of intrauterine growth retardation (IUGR) [12].

Cultural and religious norms, as well as impoverished circumstances, dictate that most Indians are strict vegetarians, while the rest have very limited meat consumption [13]. Most protein in Indian diets comes from cereal, legumes, and milk or milk products. Milk assumes a particular importance in Indian diets, because it forms the sole source of vitamin B_{12} in vegetarians and culturally accepted. Milk and milk products are an excellent source of both protein and vitamin B_{12} in vegetarians [14].

While the importance of milk during pregnancy is clear, there remains a need to quantify protein and vitamin B_{12} intakes from milk with regards to their relations with birth weight. Vitamin B_{12} and protein from milk may work together or independently with regard to promoting fetal growth. In this context, the primary objective of this study was to evaluate the relations between birth weight and maternal intake of milk, protein from milk and vitamin B_{12} from milk. The results of this study reach beyond their huge implications for public health in India to hold relevance on India's economic thought and health policy as well.

2. Subjects and methods

2.1. Study design

We used a prospective, observational cohort study design. The study was conducted at the Obstetrics and Gynecology Outpatient Department of St. John's Medical College Hospital (SMJCH) in Bangalore, India, which caters to patients of diverse socio-economic status—from urban slums to high socioeconomic status. The Institutional Ethical Review Board of SMJCH approved the experimental protocols. A written consent was obtained from each study subject at enrollment. The family member or the companion of the study participant was the witnesses and co-signed the consent form.

2.2. Subjects

The current study is a part of an observational prospective ongoing cohort study of pregnant women at St. John's Research Institute and St. John's Medical College and Hospital (SJMCH), Bangalore, India. Pregnant women (17–40 years) attending antenatal screening at the Department of Obstetrics and Gynecology at SJMCH were invited to participate in the study. Women with a clinical diagnosis of chronic illnesses such as diabetes mellitus, heart disease, hypertension, thyroid disease, those with multiple fetuses, those tested positive for HIV/Hepatitis B surface antigen/syphilis infection or those who are anticipating moving out of the city before delivery were excluded from the study. While the study is ongoing, the interim analysis on 2391 subjects consented and continued to be part of the study is represented. There were 196 fetal losses and 2195 live birth outcomes. The subjects lost to follow-up comprised of those wishing to undergo family planning (tubectomy); since SJMCH is run by Catholic Bishops, family planning is not encouraged. Therefore, the subjects who consented and had few visits at the clinic but delivered at another hospital were included in lost to follow-up. Of the 2195 live birth outcomes, first trimester diet data was not available for 159 subjects. Data from the remaining 2036 births was analyzed.

2.3. Anthropometry

Socio-demographic details were collected at baseline (11.3 ± 2.6 weeks of gestation) that included mother's age and obstetric history to classify the parity and education as a surrogate of socio-economic status. Information on maternal anthropometry, dietary intake, clinical status and blood biochemistry as per the routine antenatal care was collected at the second and third trimesters (24.2 ± 1.6 weeks and 34.1 ± 1.5 weeks, respectively) of pregnancy.

A digital balance (Soehnle, Germany) was used to record the weights of all mothers to the nearest 100 g. The digital weighing scale was standardized using the standard weights once every month. Height was measured using a stadiometer to the nearest 1 cm. Mid-upper arm circumference (MUAC) was measured to the nearest 0.1 cm using a plastic measuring tape, and skinfolds were measured at three sites (biceps, triceps and subscapular) using the Holtain Caliper (Crosswell Crymych Pembrokeshire, UK) for the assessment of body composition using prediction equations (Durnin & Womersley 1974). Weekly maternal weight gain during the second trimester (GWG_{1-2}) was calculated as the difference between the body weight at the second trimester and at baseline divided by the difference in the LMP (or gestational age) at the second trimester and at the baseline. Similarly, weekly maternal weight gain during the third trimester (GWG_{2-3}) was calculated as the difference between the weight in the third and second trimesters, divided by the difference between the LMP in the third and second trimesters, respectively. Maternal body mass index (BMI) (kg m^{-2}) was calculated using weight and height.

2.4. Routine antenatal care

Each participant was screened for routine antenatal tests (screening for HIV, VDRL and HBsAg) before enrolling in the study. The obstetrician prescribed antenatal supplements of folic acid, iron and calcium as per the antenatal schedule. Supplement compliance was recorded during the course of pregnancy in the form of tablet count. All subjects were prescribed 5 mg of folic acid per day in the first trimester with ferrous sulphate 150 mg (equivalent to 45 mg iron), folic 0.5 mg and 1000 mg calcium, each per day from the second trimester until delivery. The 1000 mg calcium prescribed per day was consumed as two tablets, each of 1250 mg calcium carbonate (equivalent to 500 mg elemental calcium) with vitamin D_3, IP 250 I.U. None of the subjects were prescribed multivitamins or multi-minerals.

2.5. Dietary intake

The dietary intake during each trimester of pregnancy was assessed using a validated food frequency questionnaire (FFQ).

The FFQ was administered be a trained research assistant at each trimester visit to obtain information about the habitual dietary intake for the preceding 3 months [15]. The nutrients and food groups were estimated for all the foods listed in the FFQ and summed to obtain the total nutrient or food group intake per day for an individual. Nutrient information was obtained for 27 macro- and micronutrients. % protein from milk products was calculated as [daily protein intake from milk products (3 g protein/100 ml of milk products)/total daily protein intake (g)] X 100 [16]. % vitamin B_{12} from milk products was similarly calculated (0.14 μg vitamin B_{12}/100 ml of milk products) [17].

2.6. Delivery and birth information

Delivery information was recorded from the medical chart pertaining to the mode of delivery, time of birth, gender of the baby, placental weight and medical condition of the mother and the baby. Birth weight (g) and length (cm) of the neonates were recorded. Infants were weighed to the nearest 10 g on an electronic weighing scale (Salter Housewares 914 Electronic Baby and Toddler Scale, NY, USA) immediately after birth. The length of the baby was measured using Infantometer (Seca 416, NY, USA). Low birth weight (LBW) was defined as a birth weight <2500 g, small for gestational age (SGA) as birth weight less than 10th percentile for gestational age and preterm delivery as delivery before 37 weeks of gestation [18].

Selection bias could influence the relations between maternal intake of milk products and birth weight as milk is a relatively expensive source of quality protein in the diet and as such, mothers with higher milk intake could also be the ones of better socioeconomic status [19]. Further, maternal socioeconomic status is a known modifier of intrauterine growth [20]. Since maternal education has been reported to be the best predictor of low birth weight amongst the various available indicators of socioeconomic status (such as maternal education, paternal occupation and family income), we chose to address this potential bias by including maternal education as a co-variate in multiple variable linear regression analyses [21].

2.7. Statistical analysis

Data were examined for normality, and normally distributed data are presented as mean ± SD or otherwise as median (quartile 1, quartile 3). Loss to follow-up was addressed by comparing baseline data of pregnancies that were lost to follow-up with those that continued to be part of the cohort. The relation of intake of milk products, % protein from milk products (%milk protein) and % vitamin B_{12} from milk products (%milk B_{12}) at each trimester with birth weight and gestational weight gain (GWG) were examined separately using linear regression. Variables identified as confounders such as gestational age at delivery, weight and height in first trimester, age, education, parity, and energy (except in the case of %milk protein and %milk B_{12}) were adjusted for in the multivariate linear regression. Because the FFQ was a three-month dietary recall, reported intake in the second trimester was considered for exploring association with weight gain from the first to second trimester $GWG_{1\text{-}2}$ (second trimester weight gain), and reported intake in the third trimester was considered for $GWG_{2\text{-}3}$ (third trimester weight gain). Two-sided P-values < 0.05 were considered statistically significant. All analyses were done using the SPSS program (version 18.0, SPSS, Chicago, IL, USA).

3. Results

Data of 2036 pregnant women with first trimester dietary data and delivery data were considered for this analysis (Fig. 1). Baseline data available among the 159 pregnancies (7.2%) that were excluded from the final analysis due to lack of trimester 1 diet data were similar to those included in the present analysis for age (24.3 ± 3.8 versus 24.4 ± 3.8 years; $P = 0.722$), height (1.55 ± 0.07 versus 1.56 ± 0.06 cm; $P = 0.073$), BMI (21.9 ± 4.4 versus 21.7 ± 3.6 kg m^{-2}; $P = 0.535$), unpaired *t*-test and parity (primiparous: 57.2% versus 59.0%; $P = 0.665$, Chi-square test). However, the educational status of the subjects excluded was significantly lower (up to high school; 42.8% versus 33.2%; $P = 0.013$, Chi-square test). The birth parameters of these 159 pregnancies were similar to the ones included in the final analysis (gestational age at birth: 38.7 ± 1.4 versus 38.6 ± 1.5 weeks; $P = 0.372$, birth weight: 2809 ± 488 versus 2876 ± 450 g; $P = 0.075$, unpaired *t*-test).

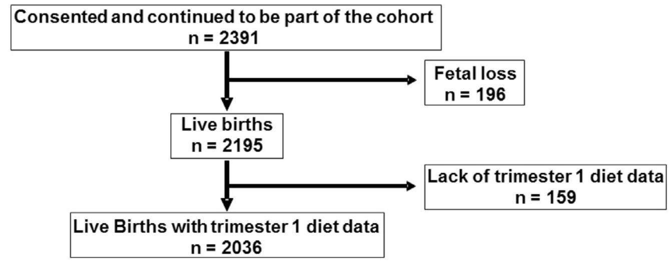

Fig. 1. Flow chart for recruitment of the St. John's birth cohort.

The anthropometric characteristics of the 2036 mothers at enrollment and of the neonates are summarized in Table 1. Mean gestational age was 38.6 ± 1.5 weeks and their mean birth weight was 2876 ± 450 g. The follow-up of the pregnancies was done till birth. 28.3% of these babies were small for gestational age (SGA), 16.9% were LBW and 9.7% were premature births. Median intake of milk products was 310 (198, 465), 417 (295, 575) and 425 (296, 592) g·day^{-1}, protein intake was 53.0 (44.0, 63.6), 62.9 (52.5, 74.0), and 63.8 (53.2, 76.1) g·day^{-1} while dietary vitamin B_{12} intake was 1.80 (1.19, 2.59), 2.32 (1.67, 3.28) and 2.43 (1.72, 3.39) μg·day^{-1} in the first, second, and third trimesters, respectively [median (quartile 1, quartile 3)]. Intake of milk products, protein and vitamin B_{12} increased significantly between the first and third trimester (Wilcoxon Signed Ranks Test: Z = −16.27, −18.16 and −16.46 respectively, all $P < 0.001$). The amount of protein derived from milk products (%milk protein) by the mothers were from 18.8% to 22.0% over the first, second and third trimesters ($P < 0.001$). The amount of vitamin B_{12} from milk ranged from 25.9% to 26.3% over the first, second and third trimesters ($P = 0.069$).

Table 1
Maternal socio-demographic characteristics and newborn characteristics (n = 2036).

Maternal Characteristics	
Age (years)[a]	24.4 ± 3.8
Weight (kg)	52.5 ± 9.5
Height (m)	1.56 ± 0.59
Gestational age at recruitment (weeks)	
Parity, n (%)	
Primiparous	1201 (59.0)
Multiparous	835 (41.0)
Education, n (%)	
Up to high school	676 (33.2)
PUC/Diploma	545 (26.8)
University and above	815 (40.0)
Newborn characteristics	
Birth weight (g)	2876 ± 450
Low birth weight, n (%)	344 (16.9)
Small for gestational age, n (%)	577 (28.3)
Gestational age at birth (weeks)	38.6 ± 1.5
Gestational weight $gain_{1\text{-}2}$ (kg·$week^{-1}$, n = 1407)	0.41 ± 0.20
Gestational weight $gain_{2\text{-}3}$ (kg·$week^{-1}$, n = 1154)	0.50 ± 0.27

[a] Mean ± SD (all such values).

To test the relation between total dietary protein intake and birth weight, we performed linear regression adjusting for gestation age at delivery. Birth weight was not associated with protein intake in all three trimesters (trimester 1- $\beta = 0.48$; $P = 0.366$, trimester 2- $\beta = 0.33$; $P = 0.604$, trimester 3- $\beta = 1.09$; $P = 0.072$). Milk is a significant source of animal protein in this predominantly vegetarian population. As such, we evaluated the association between the intakes of milk products in maternal diet with birth weight of the neonates (Supplementary Table 1). We observed an overall association of birth weight with intake of milk products in the first trimester (trimester 1- $\beta = 0.12$; $P = 0.003$, trimester 2- $\beta = 0.07$; $P = 0.132$, trimester 3- $\beta = 0.08$; $P = 0.082$). We also observed an association of birth weight with %milk protein in the first trimester (trimester 1- $\beta = 2.47$; $P = 0.004$, trimester 2- $\beta = 1.22$; $P = 0.288$, trimester 3- $\beta = 1.06$; $P = 0.367$ respectively). These associations continued to be significant in a multivariate analysis when adjusted for maternal height (m), weight (first trimester), age, parity, education, energy intake (for B_{12} and milk products only) and gestational age at birth (weeks).

We have previously reported a positive association with maternal vitamin B_{12} intake and birth weight (12). As milk is a significant source of vitamin B_{12} in this population, we tested for but failed to observe an association of birth weight with % vitamin B_{12} derived from milk (%milk vitamin B_{12}) in any trimester (trimester 1- $\beta = -0.10$; $P = 0.904$, trimester 2- $\beta = -0.71$; $P = 0.491$, trimester 3- $\beta = -0.62$; $P = 0.533$).

In an additional univariate analysis, we found that fruit intake was positively and significantly associated with birth weight in the first trimester ($\beta = 0.19$, $P = 0.002$, Supplementary Table 2). However, when we adjusted for the first trimester fruit intake in our multivariate analysis, both milk products and %milk protein remained significantly associated with birth weight in the first trimester ($\beta = 0.13$ and 2.08, $P = 0.004$ and 0.016 respectively).

We next divided the data into quintiles of intake for linear regression analysis of birth weight adjusting for gestation age at delivery. Birth weight was significantly and positively associated with intake of milk products ($P < 0.001$) in the first trimester and with %milk protein intake in all three trimesters ($P < 0.001$, Table 2). % vitamin B_{12} from milk was not associated with birth weight in any of the three trimesters.

In the multiple variable linear regression analysis of birth weight which adjusted for maternal height (m), weight (first trimester), age, parity, education, energy intake (for vitamin B_{12} and milk products only) and gestational age at birth, birth weight was positively associated with intake of milk products and of %milk protein in the first trimester ($P < 0.001$ for both) (Table 2 and Fig. 2) such that neonates born to mothers in the highest quintile of milk product or of %milk protein consumption were heavier than those born to mothers of the least quintile of consumption (87 g and 63 g respectively). No such associations were observed with %milk vitamin B_{12} in any of the three trimesters.

As gestational weight gain (GWG) is positively associated with birth weight, with the relationship being negatively affected by maternal pre-pregnancy weight, the associations of intake of milk products, %milk protein and %milk vitamin B_{12} were examined with GWG [22,23]. Though we failed to observe an association of intake in milk products in the second trimester with $GWG_{1\text{-}2}$, intake of milk products in the third trimester was associated with $GWG_{2\text{-}3}$ (One way ANOVA: $P = 0.179$ and < 0.001 respectively), such that women in the fourth and fifth quintile of milk products intake gained more weight compared to those in the first quintile of intake ($\beta = 0.12$, 95% CI: 0.06, 0.18; $\beta = 0.07$, 95% CI: 0.01, 0.13; Post-hoc Tukey's test: $P = 0.025$ and $P < 0.001$ respectively).

Intake of %milk protein in the third trimester was also positively and significantly associated with weight gain between the third and the second trimester (One-way ANOVA, $P = 0.001$). There was a significantly greater weight gain of 66 g for women in the third quintile of %milk protein intake compared to those in the first quintile ($\beta = 0.07$, 95% CI: 0.01, 0.13; Post-hoc Tukey's test: $P = 0.028$). %milk vitamin B_{12} intake was not associated with GWG either in the second or third trimester.

4. Discussion

We observed a significant association of maternal intake of milk products and of milk protein, but not vitamin B_{12} from milk with birth weight. Adequate protein intake during pregnancy is required for tissue accretion in the fetus as well as in the support tissues including the placenta [24,25]. The pregnant women in the current study are meeting the current US Dietary Reference Intakes (DRI) estimated average requirement (EAR) of 0.88 $g \cdot day^{-1}\ kg^{-1}$ for protein intake in healthy pregnant women (median intake 1.02, 1.10 and 1.04 $g \cdot day^{-1}\ kg^{-1}$ for trimester 1, 2 and 3 respectively) [26]. However, a recent report of protein requirement in healthy Canadian pregnant women using indicator amino acid oxidation method has concluded that the EARs during early and late pregnancy are 1.22 and 1.52 $g \cdot day^{-1}\ kg^{-1}$ respectively [27]. By these estimates and based on the recommendations of the Indian Council of Medical Research (1.20 $g \cdot day^{-1}\ kg^{-1}$ protein in trimester 3), the pregnant women in our study are not meeting the protein requirements [28]. Further, rodent models of maternal dietary manipulations have resulted in low birth weight both from low and very high protein intake during pregnancy [29]. However, Chong et al. reported a lack of association of maternal protein intake with offspring birth weight in a multiethnic Asian population [5]. Overall, results from human observational studies on maternal protein intake and birth weight are, at the best, inconsistent.

Some previous studies have found milk protein to be more influential on birth weight when analyzed alongside fat and carbohydrates in milk, while others still have also found that milk protein had a larger effect when compared to fat from milk [8,9]. Previous work from our center has identified milk as the major dietary source of saturated fat in this predominantly vegetarian population of pregnant mothers [30]. Though we have previously reported increased risk of intrauterine growth restriction in pregnancies with low maternal vitamin B_{12} status during pregnancy, no previous studies have undertaken an in-depth comparison between milk protein versus vitamin B_{12} from milk in relation to birth outcomes [12].

The associations of intakes of milk product and %milk protein with birth weight were strongest in the first trimester. However, we failed to observe any relations between these intakes in first or second trimester and GWG between the first and second trimester. Therefore, it is unlikely that the associations of milk products and % milk protein with birth weight are mediated by improvements in GWG. Animal and human studies implicate the peri-conceptional period as a vulnerable period for fetal development and onset of later life morbidities [31,32]. There can be at least two mechanistic explanations for these observations. One, histiotrophic transfer of nutrients via the extravillous endoglandular trophoblasts to the fetus meets the nutritional requirements of the fetus in the first trimester before establishment of the hemotrophic exchange system at the functional placenta [33]. A recent study using the model of maternal low protein diet exclusively during mouse preimplantation development has reported a depleted uterine luminal fluid amino acid composition (particularly reduced branched chain amino acids leucine, isoleucine and valine) [34]. As milk is an especially rich source of branched chain amino acids, more so in a predominantly vegetarian population like the one in the current study, it is plausible that higher milk consumption during the periconceptional period is associated with higher birth weight due to

Table 2
Simple and multiple linear regression results of birth weight with maternal intake of quintiles of milk product, % protein from milk and % vitamin B_{12} from milk intake [n = 2036 (trimester 1), n = 1386 (trimester 2) and n = 1335 (trimester 3)].

Variable	Quintile	Intake[a]	β (95% CI)[b]	$\beta_{partial}$ (95% CI)[c]
Trimester 1				
Milk products (g)	1	109.0 (61.4, 144.6)	REF	REF
	2	220.7 (197.7, 241.6)	37.3 (−15.7, 90.3)	39.2 (−12.4, 90.8)
	3	309.6 (283.9, 335.6)	45.8 (−7.1, 98.8)	47.5 (−5.5, 100.4)
	4	424.8 (389.2, 464.8)	70.1 (17.1, 123.1)*	67.5 (13.5, 121.5)*
	5	619.8 (557.7, 731.0)	84.6 (31.6, 137.6)*	86.8 (29.1, 144.6)*
%Milk protein	1	7.5 (4.5, 9.8)	REF	REF
	2	14.0 (12.8, 15.1)	45.1 (−7.9, 98.2)	38.8 (−12.6, 90.3)
	3	18.8 (17.6, 19.9)	30.6 (−22.4, 83.6)	28.2 (−23.1, 79.6)
	4	24.3 (22.9, 25.8)	73.4 (20.3, 126.4)*	63.9 (12.1, 115.7)*
	5	33.1 (30.3, 36.9)	73.9 (20.8, 126.9)*	63.1 (10.8, 115.5)*
%Milk B_{12}	1	10.4 (6.6, 13.6)	REF	REF
	2	19.7 (17.9, 21.3)	−24.6 (−77.7, 28.5)	−24.5 (−75.8, 26.9)
	3	25.9 (24.7, 27.2)	18.2 (−34.9, 71.4)	25.2 (−26.2, 76.6)
	4	31.5 (30.1, 33.1)	7.8 (−45.3, 61.0)	12.9 (−38.6, 64.4)
	5	38.6 (37.3, 39.1)	−14.8 (−68.0, 38.3)	−14.9 (−66.4, 36.6)
Trimester 2				
Milk product (g)	1	201.1 (146.3, 245.0)	REF	REF
	2	319.3 (294.7, 345.4)	34.8 (−28.5, 98.1)	36.0 (−26.0, 98.0)
	3	417.4 (390.5, 447.3)	−5.9 (−69.2, 57.3)	−12.4 (−75.7, 50.9)
	4	538.2 (509.0, 574.6)	37.9 (−25.4, 101.3)	43.3 (−22.0, 108.5)
	5	748.9 (670.2, 854.7)	35.2 (−28.1, 98.5)	28.1 (−42.3, 98.4)
%Milk Protein	1	11.6 (8.4, 13.3)	REF	REF
	2	17.4 (16.0, 18.6)	12.7 (−50.5, 75.9)	14.5 (−46.8, 75.8)
	3	22.1 (20.8, 22.8)	8.8 (−54.5, 72.0)	3.3 (−58.4, 64.9)
	4	26.3 (25.2, 27.7)	72.6 (9.4, 135.8)*	59.9 (−1.9, 121.6)
	5	33.6 (31.0, 37.8)	8.3 (−54.9, 71.5)	1.0 (−61.3, 63.3)
%Milk B_{12}	1	13.1 (8.8, 15.2)	REF	REF
	2	21.0 (19.4, 22.7)	28.0 (−35.5, 91.5)	38.1 (−23.4, 99.6)
	3	26.5 (25.4, 27.7)	−5.8 (−69.2, 57.6)	16.4 (−45.4, 78.1)
	4	31.5 (30.2, 32.9)	−16.1 (−79.6, 47.3)	8.1 (−53.6, 69.9)
	5	38.4 (36.2, 39.1)	−11.1 (−74.6, 52.4)	−10.1 (−71.7, 51.6)
Trimester 3				
Milk product (g)	1	200.8 (143.5, 247.7)	REF	REF
	2	322.5 (295.8, 350.7)	31.4 (−33.2, 96.0)	25.3 (−37.0, 87.6)
	3	425.5 (397.1, 460.8)	38.6 (−26.0, 103.3)	30.4 (−32.5, 93.4)
	4	552.8 (524.9, 591.8)	7.0 (−57.6, 71.6)	−20.3 (−85.0, 44.4)
	5	758.1 (681.6, 874.6)	32.8 (−31.9, 97.4)	5.6 (−64.3, 75.6)
%Milk Protein	1	12.0 (9.4, 13.7)	REF	REF
	2	17.6 (16.4, 18.5)	20.0 (−44.5, 84.5)	17.4 (−44.6, 79.4)
	3	22.0 (20.8, 23.2)	69.1 (4.5, 133.6)*	55.6 (−6.7, 117.9)
	4	27.0 (25.8, 28.2)	3.9 (−60.7, 68.4)	−2.9 (−65.0, 59.1)
	5	33.7 (31.2, 36.4)	27.1 (−37.5, 91.6)	17.2 (−45.4, 79.8)
%Milk B_{12}	1	11.8 (6.9, 14.8)	REF	REF
	2	20.9 (19.2, 22.6)	−0.3 (−64.9, 64.4)	−0.6 (−62.4, 61.2)
	3	26.3 (25.2, 27.5)	−11.4 (−76.0, 53.2)	3.4 (−58.7, 65.5)
	4	31.1 (30.0, 32.5)	−17.0 (−81.6, 47.7)	−3.2 (−65.3, 58.8)
	5	38.0 (35.9, 39.0)	−28.0 (−92.7, 36.6)	−30.2 (−92.3, 32.0)

* denotes $P < 0.05$.
[a] Values presented are median (quartile 1, quartile 3) in g for milk products, in % of total protein intake for %Milk protein and in % of total vitamin B_{12} intake for %Milk B_{12}.
[b] Univariate analysis adjusted for final gestational age (weeks).
[c] Multivariate analysis adjusted for final gestational age (weeks), height (m), weight (first trimester), age, parity, education and energy (for milk products only).

improved nutritional quality of the uterine glandular secretions, thereby improving the histiotrophic nutrition. Evidence in humans for this concept comes from studies on in vitro fertilized embryos. These studies suggest the importance of specific amino acids in the in-vitro growth medium for growth and viability of the embryos before transfer [35]. Two, maternal undernutrition during the peri-conceptional period likely modulates placental development as evident by a compensatory increase in the trophoectoderm endocytosis response to preimplantation period-specific maternal low protein diet in mice and by the enhanced placental weight at delivery reported in the women who were subjected to starvation only during the first trimester of pregnancy during the Dutch Hunger Winter of 1944–45 [34,36].

We observed significant association of birth weight with GWG in the second but not third trimester. On the contrary, both milk products as a whole and %milk protein showed association with GWG specifically in the third trimester. GWG comprises of both fat-free mass and fat mass. As fat-free mass but not fat mass component of GWG has been reported to be associated positively with birth weight, it is plausible that milk products and %milk protein contributed to the fat mass component of GWG and not the fat-free mass component in the third trimester [37].

We failed to observe any association of vitamin B_{12} from milk on birth weight or GWG. These results suggest that the effects of milk protein outweigh the effects of B_{12} from milk. Further, the median vitamin B_{12} intake of the pregnant women in the current study was higher than the estimated average requirements (EAR) of 1.2 μg d^{-1} even though it was lower than the recommended dietary allowance (RDA) for pregnant women (2.6 μg d^{-1}) [38]. Further, maternal vitamin B_{12} status might be a better marker than dietary vitamin

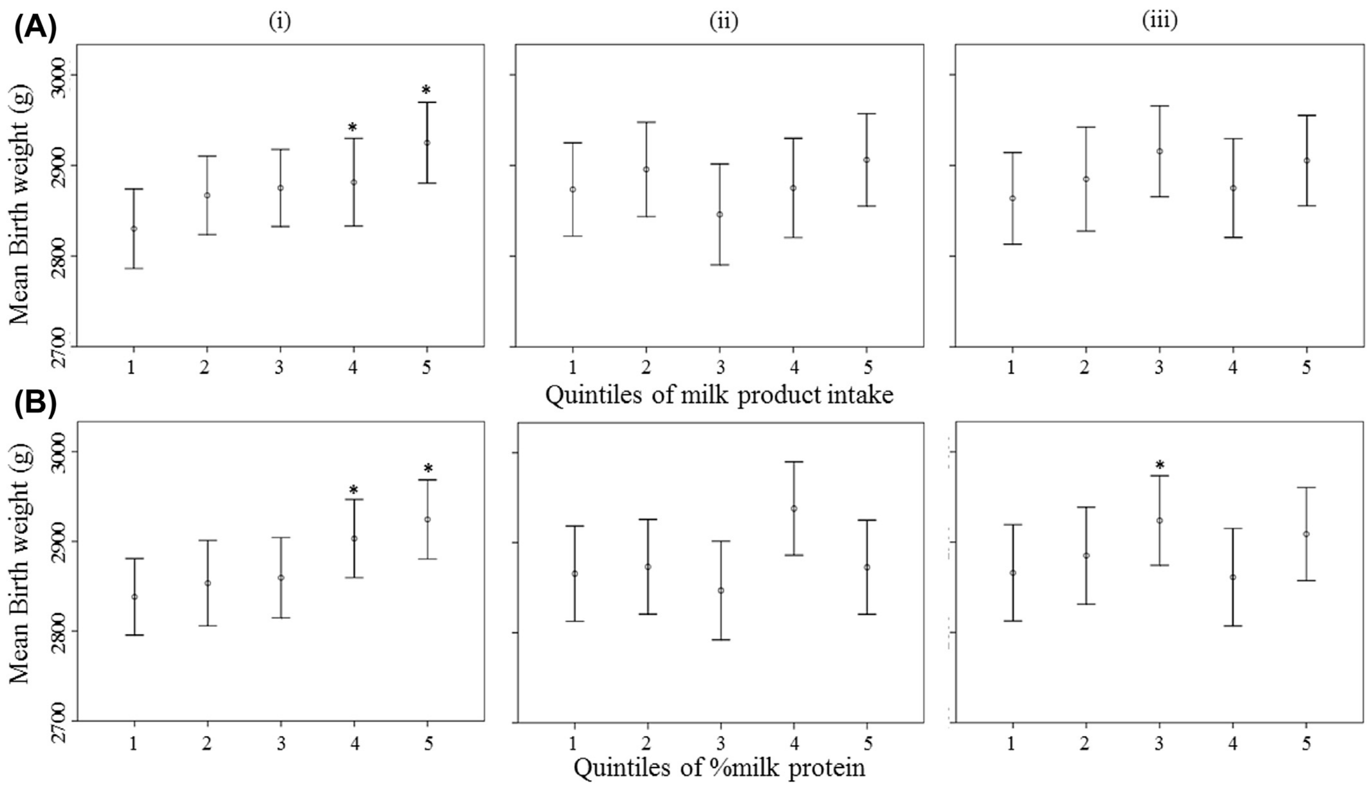

Fig. 2. Mean birth weight by quintiles of intake of milk products (A) and % milk protein (B) in the first (i), second (ii) and third (iii) trimester. Error bars denote ±2 standard error of the mean birth weight. Quintile 1 was considered as the reference quintile. * denotes $P < 0.05$.

B_{12} for its effect on fetoplacental growth. Support for this argument comes from reports of associations of birth weight predominantly with maternal vitamin B_{12} status, not dietary levels and the observations that the gut microbiome can be an effective source of vitamin B_{12} for humans [12,39].

Our statistical framework focused on the continuous variable, birth weight, as the primary birth outcome. Birth weight is the underlying concern in birth outcomes such as LBW, SGA, and pre-term babies. Therefore, it made sense for this study to examine how to increase overall birth weight instead of focusing on one outcome in particular. Furthermore, because past studies on the topic have focused on birth weight, we opted to do the same for sake of comparability. Lastly, St. John's Hospital caters to both rural and urban patients in all income groups. The ensuing diversity of our study population suggests that these results can be extrapolated to the general Indian population.

Limitations of this study include the reduction in the size of the cohort due to deliveries of cohort participants at centers other than the St. John's Hospital. Another limitation is the drop in birth weight in the fifth quintile of intake in the second trimester or the fourth and fifth quintile of intake in the third trimester when examining %milk protein. The reason for this dip has yet to be fully determined. However, some possible causes have been examined. This data set examined all babies—without eliminating those who are premature or low birth weight. Another possible explanation is that too much milk protein has negative effect on fetoplacental growth.

Any possible solution to the problem of poor birth outcomes must begin with the mother. Ensuring that women and adolescent girls get sufficient milk as part of a healthy diet could be the answer to resolving low birth weight issues. India's public health agenda needs to shift toward guaranteeing the health of women and girls beginning in adolescent years. India's rate of childhood stunting is one of the highest in the world, and thus, bigger babies need to be accompanied by a larger childbearing population [40].

The health benefits of a policy change that ensures adequate milk intake before and during pregnancy are obvious given the results of the current and older studies; less realized are the economic benefits to India of such a policy. In the most general terms, given that most of health care expenditures are out-of-pocket, preventive measures such as ensuring sufficient milk intake could ease the financial burden on many families—more than 30% of which live below the poverty line [41,42]. More specifically, in the short term, preventive measures could save money on neonatal intensive care unit (NICU) costs. The amount of money that may be spent on risk factors later in life caused by LBW makes such a policy beneficial in the long run as well.

Disclosures/Conflict of interest

The authors have no potential conflicts of interest relevant to this article.

Authors' contributions to the manuscript

AM, PD, TT, SB and AVK- designed research; PD, SD and AT-conducted research; AM and SB— analyzed data and wrote manuscript; TT and AVK- edited the manuscript; AVK-had responsibility for the final content; AM, PD, SB, SD, AT, AVK and TT-read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnesp.2018.03.125.

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