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Effects of a dairy product fortified with multiple micronutrients and omega-3 fatty acids on birth weight and gestation duration in pregnant Chilean women

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Abstract

Objective: To test the hypothesis that maternal food fortification with omega-3 fatty acids and multiple micronutrients increases birth weight and gestation duration, as primary outcomes.

Design: Non-blinded, randomised controlled study.

Setting: Pregnant women received powdered milk during their health check-ups at 19 antenatal clinics and delivered at two maternity hospitals in Santiago, Chile.

Subject: Pregnant women were assigned to receive regular powdered milk ($n = 477$) or a milk product fortified with multiple micronutrients and omega-3 fatty acids ($n = 495$).

Results: Intention-to-treat analysis showed that mean birth weight was higher in the intervention group than in controls (65.4 g difference, 95% confidence interval (CI) 5–126 g; $P = 0.03$) and the incidence of very preterm birth (<34 weeks) was lower (0.4% vs. 2.1%; $P = 0.03$). On-treatment analysis showed a mean birth weight difference of 118 g (95% CI 47–190 g; $P = 0.001$) and a relative fall in both the proportion of birth weight ≤3000 g ($P = 0.015$) and the incidence of pre-eclampsia ($P = 0.015$). Compliance with the experimental product was apparent from a haematological study of red-blood-cell folate at the end of pregnancy, which was performed in a sub-sample. In both types of analyses, positive differences were also present for mean gestation duration, birth length and head circumference. Nevertheless, the relatively small sample sizes allowed a statistical power of >0.80 just for mean birth weight and birth length in the on-treatment analysis; birth length in that analysis had a difference of 0.57 cm (95% CI 0.19–0.96 cm; $P = 0.003$).

Conclusions: The new intervention resulted in increased mean birth weight. Associations with gestation duration and most secondary outcomes need a larger sample size for confirmation.

Keywords
Maternal nutrition
Food supplementation
Multiple micronutrients
Omega-3 fatty acids
Birth weight
Gestation duration

The potential benefits of providing micronutrients to pregnant women, either through tablets or fortified food products, are now under investigation[1-5]. A supplement containing the recommended dietary allowances of 15 different micronutrients has been proposed[6], supported by the findings of randomised controlled trials among HIV-positive women in Tanzania[7] and HIV-negative women in Nepal[8,9]. The Nepal studies showed improvements in mean birth weight of 64 g and 77 g in infants born to women supplemented with multiple micronutrients as daily tablets compared with controls receiving either vitamin A or folic acid–iron, respectively[8,9]. The former study found that multiple micronutrients supplementation also reduced the risk of low birth weight by

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14% but that finding did not have additional benefit compared with supplementing with folic acid–iron[8]. For the last 6 years Chile has been supplying these two micronutrients, i.e. folic acid and iron, to the general population of pregnant women through the fortification of white flour and the delivery of ferrous sulphate tablets, respectively[10,11]. Therefore, the possible impact of a combination of multiple micronutrients can be further explored as public programmes already provide folic acid and iron. The improvements in birth weight mentioned above are similar to those achieved in our earlier randomised controlled trial of a multiple micronutrient-fortified milk-based supplement, in which we observed a 73 g increase in the experimental group at a time when iron tablets delivery and folic acid fortification were not public programmes[12]. On the other hand, several studies have shown that the consumption of either omega-3 fatty acids or fish increases birth weight and prolongs gestation[3,4].

In the present paper we report the results of a trial in which, in addition to vitamins and minerals, an omega-3 fatty acid (α-linolenic acid) and an omega-6 fatty acid (linoleic acid) were added to the powdered milk. This trial took advantage of the fact that the Chilean Ministry of Health initiated powdered milk delivery to pregnant women in 1954. That measure has encouraged women to use the public health system; 99.9% of all pregnant women in Chile have regular antenatal check-ups and deliver their babies at a maternity hospital[13]. On the basis of our previous randomised trial[12] and recent evidence of the effect of omega-3 fatty acids on birth weight and gestation duration[3,4], it was expected to prove the hypothesis that supplementation with this milk-based product increases birth weight and can prolong gestation duration.

Methods

Population

The sampling frame for the trial included all pregnant women attending 19 urban health clinics (each covering a population of around 40 000) belonging to the Servicio de Salud Metropolitano Sur-Oriente (Southeast Metropolitan Public Health Service), Santiago, Chile (total population 5 million). Women attending these clinics come from mainly low-income, ethnically mixed families (Amerindian and Hispanic) and deliver their babies at two maternity hospitals. Recruitment criteria were maternal age 18 years and over, parity 0–5, up to 20 weeks' gestation according to the date of the last menstrual period and confirmed by ultrasound, non-consumers of drugs and alcohol, and underweight (body mass index (BMI) $\leq 21.2\,kg\,m^{-2}$ at 10 weeks' gestation, as defined by Chilean charts for pregnant women[13]). Women with multiple pregnancies or suffering from chronic diseases that could affect foetal growth were excluded. Smokers and women with diseases that were diagnosed during pregnancy were not excluded from this study. When a pregnant woman met the recruitment criteria, the midwife in charge explained to her the nature of the research without giving explicit clues as to expectations of the study and marked her medical record and her take-home booklet to indicate that she had been included in the study.

Two ethics committees reviewed and approved this research, one from the Faculty of Medicine, Pontifical Catholic University of Chile and the other from Southeast Metropolitan Public Health Service at the Sótero del Río Hospital.

Procedures

Midwives in charge assigned the women in their initial pregnancy visit using the order of arrival: odd numbers to the experimental group and even numbers to the control group. This alternate allocation has been referred to as systematic sampling[14]. Women in the control group received 2 kg per month of the regular powdered milk (Purita Fortificada® or product PF, produced in Chile by different companies) that is fortified with small amounts of iron sulphate, copper, zinc and vitamin C (Table 1). Women in the experimental group received 2 kg per month of powdered milk (Maman® or product M, produced by Parmalat SpA, Parma, Italy), fortified with multiple micronutrients and both α-linolenic acid (omega-3 fatty acid) and linoleic acid (omega-6 fatty acid); iron was supplied in an amino-chelated form (Table 1).

The study could not be blinded because Chilean regulations do not allow delivery of food without information on its composition. Good care was taken so as not to disclose the expectations of the study in favour of one or the other supplement. That information was not known by either midwives or the pregnant women enrolled in the study. For those co-authors who performed the calculations, groups were simply labelled 1 and 2, and the coding was not known by them either. These measurements should suffice to minimise the possible effects of non-blinding.

Food supplements were provided in similar take-home cardboard packages of 1 kg of each product with different labels indicating the brand names and chemical composition. Participants received the products from enrolment to delivery. They were recommended to consume 66 g daily in three cups diluted to 10% in water. Booklets with instructions and suggestions for the preparation and consumption of the products were given to each woman.

The follow-up plan included two home visits by nutritionists at weeks 20 and 35 of gestation to perform 24-hour dietary recall and socio-economic surveys. Mean values of the nutrients from the two surveys were calculated to estimate the daily amounts provided by the home

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Table 1 Chemical composition of powdered milks per 100 g: regularly delivered product PF (Purita Fortificada®) and newly produced product M (Mamán®)

	PF	M
Energy (kcal)	498.0	521.0
Protein (g)	28.0	25.0
Fats (g)	26.0	21.0
Milk fat	26.0	10.5
Vegetable fat		10.5
Polyunsaturated fatty acids		5.3
Omega-3 fatty acids		0.9
Omega-6 fatty acids		4.4
Carbohydrates (g)	38	58
Lactose	38	3
Vitamins		
A (μg)	278	1200
Thiamin (B_1) (mg)	0.3	1.0
Riboflavin (B_2) (mg)	1.2	1.0
Pyridoxine (B_6) (mg)	0.3	2.0
B_{12} (μg)	3.2	1.5
C (mg)	70	110
D_3 (μg)	30	15
E (mg)	0.35	45
Niacin (PP) (mg)	0.830	10
Biotin (μg)	–	45
Folic acid (μg)	36.7	600
Pantothenic acid (mg)	2.3	–
Minerals		
Ca (mg)	905	960
P (mg)	770	720
Mg (mg)	10.5	90
Zn (mg)	5	12
Fe (mg)	10	27
Bioavailable Fe (mg)	1	4.5
Cu (mg)	0.5	–
Se (μg)	–	15

diet and from the supplements. The follow-up plan also included check-ups by the midwives: one per month during the first and second trimester and at least two per month during the third trimester. During these visits the women's body weights and heights were recorded in a standardised manner using beam-balance scales (Condor Corporation). Blood pressure was determined using calibrated sphygmomanometers, with the women seated and having rested for at least 15 min.

As part of routine care, blood samples were taken for determination of blood group, rhesus status and glycaemia and rapid plasma reagin test for syphilis. The latter test was repeated at week 28 of gestation and glucose intolerance was also assessed at week 28 in women with risk factors for gestational diabetes. Urine samples for testing the sediment in all women and for testing the presence of protein in hypertensive women were undertaken at recruitment and also as part of the regular procedures at the health clinics. The diagnosis of pre-eclampsia was based on hypertension, oedema and proteinuria, confirmed by laboratory analysis of a 24-hour urine collection (>300 mg was considered abnormal). In addition, plasma urea concentration and creatinine clearance were measured. All women found to have significant pathology were referred for appropriate care.

Gestational age was estimated according to the date of the last menstrual period and confirmed by transvaginal ultrasound foetal biometry during the first trimester and transabdominal ultrasound during the second trimester. We used either a Voluson 730 PRO (GE Healthcare) or an Acuson 120XP (Acuson Inc.) instrument. In pregnancies less than 13 weeks and 6 days, we used crown–rump length and Robinson's chart[15]. Between 14 and 20 weeks, we used biparietal diameter and head circumference and the charts of Chitty[16]. Trained ultrasonographers at each of the two maternity hospitals of the Southeast Metropolitan Public Health Service performed the scans.

Mother and infant morbidity was diagnosed and registered by physicians in charge. Infants were dried and weighed on an electronic self-calibrating scale immediately after delivery, either a Tanita 1583 electronic scale accurate to 10 g or a Seca 345 electronic scale accurate to 20 g. Crown–heel length was measured on a custom-made neonatometer, and head circumference was measured with an insertion tape, both to the nearest mm. Adequacy of size for gestational age was estimated using the Aberdeen standard[17]. Newborns below the 10th percentile were classified as foetal growth-restricted (FGR) infants. Mothers were weighed, 24–48 h after delivery.

Socio-economic level was determined using labour and education combined[18]; the resulting classification has been correlated to adult nutritional status in Chile[19]. Sanitary conditions, i.e. connection to potable water and sewage, were also studied.

We defined loss to follow-up as failure to attend the antenatal clinic for 3 months or failure to locate the participant after three home visits.

Statistical analysis

Pregnant women were enrolled in the study between 14 May 2002 and 28 February 2003. The follow-up period ended by November 2003. Primary outcomes were birth weight and gestational duration means. Secondary outcomes included mean infant crown–heel length, head circumference, and incidences of maternal pre-eclampsia, pre-term deliveries (<34 weeks and <37 weeks), low birth weight categories (<2500 g and <3000 g) and FGR infants. We calculated the sample size to reach a power of 0.80 and a two-sided significance level of 0.05. At 319 participants per group the study would attribute significance to a change in mean birth weight of 100 g (assuming a control mean of 3100 ± 450 g) and a change in mean gestational duration of 3 days (assuming a control mean of 275 ± 14 days). We allowed for the possibility of 40% loss to follow-up[12]. The sample size was thus estimated at 532 women in each group.

Microsoft® Excel was used for data management and SPSS version 13.0 for statistical analysis. Student's *t*-test for independent samples and the χ^2 test were used to

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calculate the statistical significance of the differences between means and proportions, respectively. Differences in mean values with 95% confidence interval (CI) were calculated to estimate the magnitude of effects related to primary and secondary outcomes. Crude odds ratios (ORs) with 95% CI were calculated to estimate risk reduction of categorical primary and secondary outcomes. Multiple linear regression models were fitted to estimate the effect of the milk supplementation on birth weight after adjustment for other covariates. We assessed categorical outcomes using logistic regression analyses with adjustment for other covariates. Effects with *P*-values equal to or below 0.05 were considered statistically significant. For all comparisons, the power of the statistical test was expected at 0.80[20].

Dietary surveys were analysed using Food Processor II (Esha Research), previously validated for Chilean food[21]. Diet adequacy for micronutrients was calculated according to current recommendations from the US Food and Nutrition Board[22–25]; the so-called Dietary Reference Intakes (DRIs).

Haematological study

In order to ascertain whether the women in the study actually consumed the new food product, a haematological study was performed on a sub-sample of the experimental and control groups. All women recruited during three months – May, June and July 2003 – in four of the 19 clinics were informed and asked for written consent to participate in a haematological study aimed to determine red-blood-cell (RBC) folate concentration. This is a reliable reference method to estimate compliance with a chronically consumed folic acid-fortified food[26]. Blood samples were collected from the antecubital vein after an overnight fast at 10–19 and 35–36 weeks of gestation. Blood samples were immediately placed on ice and transported to the laboratory to be processed. The measurement of RBC folate was done using an ion capture assay with the Axsym System (Abbott Laboratories).

Results

A total of 1173 women were considered eligible, and they were recruited and randomised (Fig. 1). Some cases did not meet the inclusion criteria and were not further analysed. Among women who discontinued the trial (Fig. 1), there were no significant differences between the groups except that gestational age at recruitment was lower in group M (Table 2).

Due to irregularities in the supply of product M for three months, women recruited during those months did not receive treatment as allocated. Specifically, the manufacturer added vanilla in excess to product M, causing mothers to reject the product according to reports from the midwives and nutritionists that were confirmed by the authors. For that reason, analyses per protocol ('on treatment') and by intention to treat are reported.

Women included in the intention-to-treat analyses were 83% of all eligible and randomised women. Because women who did not receive the treatment as allocated during the three months were excluded from on-treatment analyses, the previous 17% loss to follow-up reached 40.5%. There were no stillbirths or neonatal deaths during the study. Differences in baseline variables between women who did not receive treatment as allocated and the rest of the women who were on treatment were negligible (Table 3).

Per-protocol or on-treatment analysis

Selected baseline biological and social variables were similar between the control and the experimental groups with the exception of gestational age at recruitment, which was slightly higher in group M (Table 4). This difference probably arises from the inverse situation observed in Table 2 and it is further analysed below when multiple regression outcomes are presented. Socio-economic control variables in Table 4 show that sanitary conditions of households were similar and met desired standards with a few exceptions. The distributions of women in terms of the socio-economic ESOMAR (European Society for Opinion and Marketing Research) classification showed that 94.5% of the PF group and 94.1% of the M group were in the medium, medium–low and low socio-economic levels. The proportion of women with 8 years of education or more was greater than 80% in both groups.

Mean daily consumption of the two supplements was slightly higher in the control group ($36.9 \pm 26.2\,g\,day^{-1}$) than in the experimental group ($31.2 \pm 31.0\,g\,day^{-1}$). Table 5 shows that estimated daily intakes of micronutrients were significantly higher in group M with power of >0.80 for omega-3 fatty acid and vitamins B_6, E, niacin, biotin and folic acid, and minerals zinc and iron. On the other hand, copper and vitamin D_3 were significantly higher in group PF. We assessed the adequacy of dietary intakes of each vitamin and mineral by calculating the mean intake as a percentage of the DRI. Mean intakes of iron, vitamin B_6 and biotin had mean adequacy figures below 90% (74%, 84% and 64%, respectively) in the PF group. Mean intake of magnesium was 81% and 76% of DRI in the PF and M groups. All other vitamins and minerals studied were more than 90% adequate in the M group.

Mean birth weight, birth length, head circumference and gestational age at birth were significantly higher in group M; however, only the tests for birth weight and birth length had power of >0.80 (Table 6). A multiple linear regression analysis of the effect on birth weight

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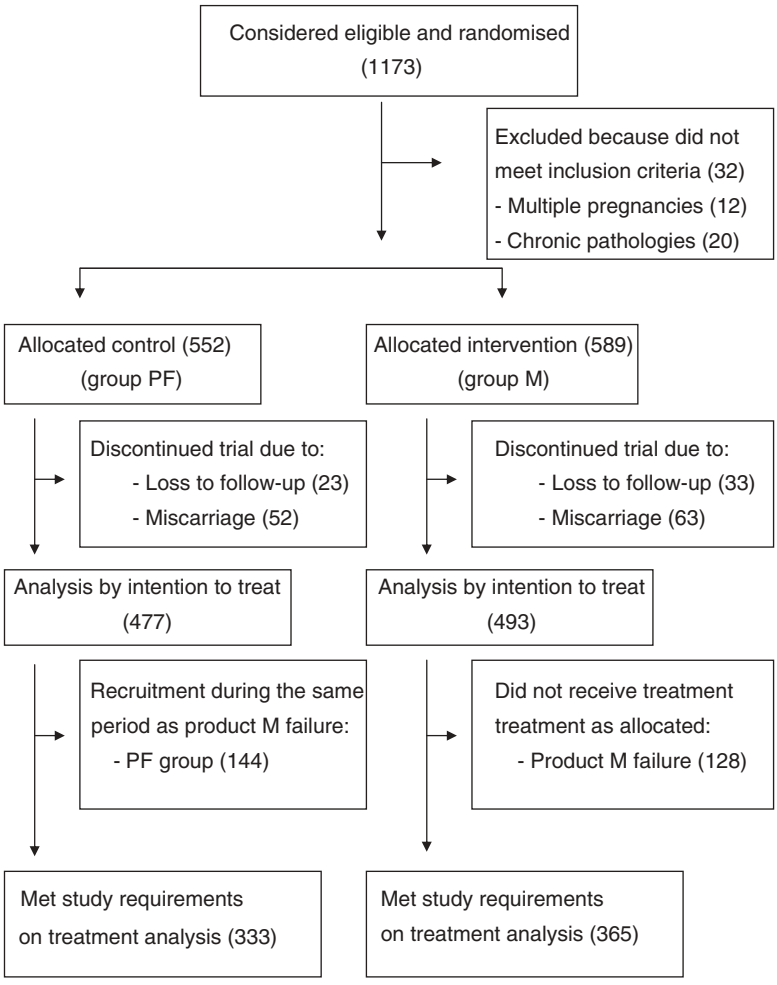

Fig. 1 Attrition and composition of each study group (*n*)

Table 2 Mean (±standard deviation) baseline variables at the beginning of pregnancy in women who discontinued the trial in the two study groups

Variable	PF group ($n = 75$)	M group ($n = 96$)	*P*-value
Maternal height (cm)	157.08 ± 6.16	157.50 ± 6.54	0.670
Gestational age at recruitment (weeks)	11.11 ± 4.30	9.70 ± 3.13	0.016
Weight at recruitment (kg)	50.11 ± 4.38	49.64 ± 4.75	0.509
Maternal age (years)	25.11 ± 7.45	25.06 ± 5.73	0.957
Parity	1.88 ± 1.05	1.89 ± 1.10	0.907
Body mass index (kg m^{-2})	20.35 ± 1.48	20.02 ± 1.30	0.121

of maternal age, height, parity, BMI at recruitment and gestational age at recruitment, and the dairy product delivered, showed that only the intervention group and BMI at recruitment had statistically significant effects ($P = 0.001$ and 0.020, respectively).

The incidence of adverse pregnancy outcomes, including preterm deliveries (both <34 weeks and <37 weeks), pre-eclampsia, birth weight ≤3000 g, birth weight ≤2500 g and FGR infants, were lower in the experimental (M) group. Significant statistical differences were attained for pre-eclampsia, birth weight ≤3000 g and FGR infants; none of those differences had a power of >0.80 (Table 7). Adjusted ORs were similar to crude ORs; covariates used were from the recruitment period: maternal age, weight and height, gestational age and parity. Rates of instrumental delivery and Caesarean section, and birth asphyxia (Apgar score <7 and <4), were similar in the two groups (Table 7). Rates of complications of pregnancy (pathological outcomes that appeared during gestation) and smokers (at least one cigarette per day) were also similar in the two groups.

Cross-tabulation of the combined study groups showed that the incidence of pre-eclampsia was similar in primiparae and multiparae, i.e. 2.4% and 3.6%, respectively

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Table 3 Mean (±standard deviation) baseline variables at the beginning of pregnancy (group 1 – women who did not receive treatment as allocated; group 2 – women who were on treatment)

Variable	Group 1 ($n = 272$)	Group 2 ($n = 698$)	*P*-value
Maternal height (cm)	157.19 ± 5.76	157.08 ± 5.89	0.793
Gestational age at recruitment (weeks)	10.72 ± 3.39	11.03 ± 3.42	0.204
Weight at recruitment (kg)	49.55 ± 4.07	49.34 ± 4.29	0.508
Maternal age (years)	24.17 ± 5.30	23.95 ± 5.14	0.572
Parity	1.74 ± 0.91	1.70 ± 0.91	0.539
Body mass index (kg m^{-2})	20.04 ± 1.14	19.98 ± 1.19	0.477

Table 4 Mean (±standard deviation) selected biological and socio-economic baseline variables in the control (PF) and experimental (M) groups: on-treatment analysis

Variable	PF group ($n = 333$)	M group ($n = 365$)	*P*-value
Maternal height (cm)	157.06 ± 5.94	157.11 ± 5.86	0.909
Gestational age at recruitment (weeks)	10.66 ± 3.39	11.36 ± 3.41	0.006
Weight at recruitment (kg)	49.33 ± 4.19	49.36 ± 4.39	0.919
Maternal age (years)	24.12 ± 5.12	23.80 ± 5.17	0.415
Parity	1.74 ± 0.91	1.68 ± 0.90	0.392
Primigravid (%)	45.2	51.4	0.105
Male newborns (%)	48.93	50.13	0.754
Education >8 years (%)	80.70	82.10	0.630
Connected potable water at home (%)	99.10	99.20	0.910
Connected sewage disposal at home (%)	99.40	99.20	0.729
ESOMAR classification (%)			
AB (High level)	0.3	0.5	0.856
CA (medium–high level)	4.2	4.4	0.955
CB (medium level)	33.4	34.9	0.736
D (medium–low level)	44.6	40.4	0.296
E (low level)	17.5	19.8	0.496

ESOMAR – European Society for Opinion and Marketing Research.

Table 5 Mean (± standard deviation) of selected nutrients* in the control (PF) and experimental (M) groups: on-treatment analysis

Variable	PF group ($n = 333$)	M group ($n = 365$)	*P*-value (power)
Energy (kcal)	2258.87 ± 598.96	2229.16 ± 561.45	0.500 (0.10)
Protein (g)	80.77 ± 23.59	81.07 ± 31.17	0.888 (0.05)
Carbohydrates (g)	345.61 ± 98.71	343.85 ± 93.45	0.810 (0.59)
Total fat (g)	64.45 ± 22.53	62.12 ± 20.20	0.153 (0.31)
Omega-3 (g)	0.57 ± 0.30	0.90 ± 0.52	<0.001 (>0.99)
Omega-6 (g)	16.56 ± 6.88	18.63 ± 17.06	0.033 (0.62)
Vitamin A (μg)	971.07 ± 1074.70	844.36 ± 809.47	0.081 (0.46)
Vitamin B_1 (mg)	2.52 ± 0.78	3.10 ± 7.94	0.166 (0.19)
Vitamin B_2 (mg)	2.36 ± 0.77	2.23 ± 0.67	0.018 (0.66)
Vitamin B_6 (mg)	1.64 ± 0.66	2.14 ± 1.21	<0.001 (>0.99)
Vitamin B_{12} (μg)	4.54 ± 7.05	3.86 ± 6.06	0.179 (0.28)
Vitamin C (mg)	116.38 ± 63.09	121.35 ± 70.87	0.327 (0.17)
Vitamin D (μg)	12.50 ± 7.73	7.34 ± 5.86	<0.001 (>0.99)
Vitamin E (mg)	20.58 ± 8.71	33.69 ± 16.38	<0.001 (>0.99)
Niacin (mg)	24.67 ± 7.35	27.19 ± 8.38	<0.001 (>0.99)
Biotin (μg)	19.27 ± 9.71	33.37 ± 16.30	<0.001 (>0.99)
Folic acid (μg)	817.52 ± 201.26	971.59 ± 255.23	<0.001 (>0.99)
Ca (mg)	924.83 ± 332.82	946.72 ± 362.55	0.406 (0.13)
Mg (mg)	265.63 ± 85.75	282.09 ± 87.27	0.012 (0.74)
Zn (mg)	10.58 ± 3.22	12.77 ± 4.87	<0.001 (>0.99)
Fe (mg)	20.06 ± 8.03	24.30 ± 9.76	<0.001 (>0.99)
Cu (mg)	1.78 ± 0.84	1.51 ± 0.67	<0.001 (>0.99)
Se (μg)	139.73 ± 46.98	144.34 ± 48.56	0.204 (0.252)

* Home diet and products PF and M included.

($P = 0.350$). Results were similar in each study group. Maternal weight gain during pregnancy and weight loss after delivery were lower in the control group but the differences were not statistically significant (14.00 ± 4.97 vs. 14.53 ± 4.78 kg, $P = 0.152$; and 4.89 ± 2.39 vs. 5.03 ± 2.76 kg, $P = 0.483$).

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Table 6 Mean (±standard deviation) of primary and secondary outcomes: on-treatment and intention-to-treat analyses

Variable	On-treatment analysis				Intention-to-treat analysis			
	PF group ($n = 333$)	M group ($n = 365$)	Difference (95% CI)	*P*-value (power)	PF group ($n = 477$)	M group ($n = 493$)	Difference (95% CI)	*P*-value (power)
Birth weight (g)	3174.13 ± 507.78	3292.41 ± 448.12	118.28 (46.82, 189.74)	0.001 (0.91)	3200.47 ± 506.02	3265.87 ± 452.09	65.40 (4.87, 125.93)	0.034 (0.61)
Gestation duration (days)	271.65 ± 12.55	273.52 ± 9.96	1.86 (0.17, 3.56)	0.030 (0.69)	272.00 ± 12.54	273.40 ± 9.77	1.40 (−0.02, 2.82)	0.054 (0.61)
Infant length (cm)	48.83 ± 2.69	49.40 ± 2.42	0.57 (0.19, 0.96)	0.003 (0.85)	48.98 ± 2.62	49.36 ± 2.30	0.37 (0.06, 0.68)	0.019 (0.71)
Head circumference (cm)	34.19 ± 1.78	34.48 ± 1.75	0.29 (0.03, 0.56)	0.029 (0.59)	34.25 ± 1.72	34.45 ± 1.68	0.20 (−0.02, 0.41)	0.069 (0.46)

CI – confidence interval.

The compliance haematological study of women in the on-treatment groups showed that mean RBC folate concentration in the experimental group ($n = 77$) was similar to that in the control group ($n = 65$) at the beginning of pregnancy: 472 ± 124 vs. 480 ± 109 ng ml^{-1}, respectively ($P = 0.652$). However, at the end of pregnancy it was significantly higher: 635 ± 143 vs. 584 ± 136 ng ml^{-1}, respectively ($P = 0.031$).

Intention-to-treat analysis

In the comparisons by intention to treat, the baseline variables closely resembled the values presented in Table 4 for the on-treatment study groups. Mean daily consumption of the two supplements was slightly higher in the control group (36.3 ± 26.5 g day^{-1}) than in the experimental group (32.0 ± 30.1 g day^{-1}), as in the on-treatment analysis. The estimated daily intakes of macro- and micronutrients and the *P*-values for the differences between groups were similar to those presented in Table 5.

Mean values of primary and secondary outcomes are presented in Table 6. Mean birth weight and infant length were significantly higher in the experimental group; mean birth weight in newborns of mothers with BMI at recruitment ≥20.2 kg m^{-2} was also significantly higher (Table 6).

Adjusted ORs were similar to crude ORs presented in Table 7; with the exception of pre-term deliveries <34 weeks, other secondary outcomes had non-significant *P*-values with associated power of <0.80. Rates of instrumental delivery and Caesarean section, plus Apgar scores <7 and <4, were similar in the two groups. Complications of pregnancy (adverse outcomes that appeared during gestation) and smokers of at least one cigarette per day were similar in the two groups. The proportions of all adverse outcomes were lower in the experimental group; however, only preterm deliveries <34 weeks were significantly lower but just reaching a power of 0.67 (Table 7).

Maternal weight changes during pregnancy and after delivery were similar to those in the on-treatment analysis group.

Discussion

We have shown that antenatal supplementation with a milk product fortified with multiple micronutrients and omega-3 fatty acid was associated with increased birth weight and birth length when compared with powdered milk that was slightly fortified. Gestational duration, also a primary outcome in this study, had significant *P*-values in both analyses (i.e. on-treatment and intention-to-treat) despite the fact that the statistical power reached 0.69 and 0.61 in both cases. It is expected that if the observed difference were maintained in a larger sample size, the

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Table 7 Frequencies (%) of categorical values for primary and secondary outcomes and frequencies (%) of pregnancy complications and smokers: crude odds ratios, on-treatment and intention-to-treat analyses

	On-treatment analysis			Intention-to-treat analysis		
Variable	PF group ($n = 333$)	M group ($n = 365$)	Crude OR (95% CI) [*P*-value; power]	PF group ($n = 477$)	M group ($n = 493$)	Crude OR (95% CI) [*P*-value; power]
Preterm <34 weeks	8 (2.4)	2 (0.5)	4.47 (0.87, 30.66) [0.054; 0.57]	10 (2.1)	2 (0.4)	5.26 (1.08, 34.90) [0.020; 0.67]
Preterm <37 weeks	21 (6.3)	16 (4.4)	1.47 (0.72 to 3.01) [0.257; 0.20]	32 (6.7)	22 (4.5)	1.54 (0.85, 2.79) [0.127; 0.32]
Pre-eclampsia	15 (4.5)	6 (1.6)	2.82 (1.01, 8.25) [0.027; 0.61]	16 (3.4)	8 (1.6)	2.10 (0.84, 5.41) [0.083; 0.44]
Birth weight <2500 g	28 (8.4)	20 (5.5)	1.58 (0.84, 2.99) [0.127; 0.33]	37 (7.8)	27 (5.5)	1.45 (0.84, 2.50) [0.153; 0.30]
Birth weight <3000 g	106 (31.8)	86 (23.6)	1.51 (1.07, 2.15) [0.015; 0.68]	141 (29.6)	123 (24.9)	1.26 (0.94, 1.69) [0.107; 0.38]
FGR infants	29 (8.7)	15 (4.1)	2.23 (1.13, 4.44) [0.013; 0.70]	39 (8.2)	30 (6.1)	1.37 (0.82, 2.32) [0.205; 0.25]
Instrumental delivery (forceps)	7 (2.1)	9 (2.5)	0.85 (0.28, 2.52) [0.497; 0.05]	7 (1.5)	10 (2.0)	0.72 (0.25, 2.07) [0.561; 0.09]
Caesarean section	21 (6.3)	14 (3.9)	1.69 (0.80, 3.57) [0.203; 0.31]	22 (4.6)	17 (3.4)	1.35 (0.68, 2.71) [0.429; 0.16]
1-min Apgar score <7	17 (5.1)	13 (3.6)	1.44 (0.65, 3.21) [0.326; 0.16]	21 (4.4)	20 (4.1)	1.08 (0.56, 2.11) [0.804; 0.04]
5-min Apgar score <7	0	1 (0.3)	Not estimated	1 (0.2)	2 (0.4)	0.51 (0.02, 7.19) [0.579; 0.01]
1-min Apgar score <4	3 (0.9)	4 (1.1)	0.81 (0.14, 4.32) [1.000; 0.04]	5 (1.1)	7 (1.4)	0.73 (0.20, 2.58) [0.773, 0.06]
5-min Apgar score <4	0	1 (0.3)	Not estimated [1.000; –]	0	1 (0.2)	Not estimated [1.000; –]
Complications of pregnancy*†	25 (7.5)	20 (5.5)	Not estimated [0.276; 0.19]	28 (5.9)	23 (4.7)	Not estimated [0.401; 0.13]
Smokers	27 (8.1)	35 (9.6)	Not estimated [0.492; 0.10]	47 (9.9)	49 (9.9)	Not estimated [0.964; –]

OR – odds ratio; CI – confidence interval; FGR – foetal growth-restricted.
* Complications registered in the on-treatment analysis were13 cases of intrahepatic cholestasis of pregnancy; two cases with pyelonephritis; one case of hyperemesis gravidarum; one case of hypothyroidism; 21 cases of pre-eclampsia; two cases of syphilis; two cases of cholelithiasis; and three cases of drug addiction.
† Complications registered in the intention-to-treat analysis were 14 cases of intrahepatic cholestasis of pregnancy; two cases with pyelonephritis; three cases of hyperemesis gravidarum; one case of hypothyroidism; 24 cases of pre-eclampsia; two cases of syphilis; two cases of cholelithiasis; and three cases of drug addiction.

power would be increased in both cases; 450 women in each branch would be needed to reach a power of 0.80. However, owing to the product failure encountered, this larger sample size could not be reached. Most differences are more accentuated in the on-treatment analysis, possibly suggesting that a larger sample size would have meant results with enough power in the other secondary outcomes; most of them had statistically significant differences.

Other important limitations of the present study were the impossibility of a blind design due to legal constraints; a slight difference in gestational age at recruitment; and also the fact that, under the conditions of the study, it was impossible to have strict control of compliance with the prescribed amounts of the products taken to their homes by women randomly assigned to the one or the other product. We believe, however, that the possible effect of these factors was negligible. We have explained earlier that the hypothesis of the study was not made explicit to the pregnant women or to the persons involved in collecting data or performing calculations. As for the effect of gestational age, including it as a covariate in the regression analyses enabled it to be controlled for. Finally, women apparently consumed the new food product M as indicated by the results from the haematological study. As far as bioavailability has not been well established, it is not possible to estimate actual exact consumption of product M from this observation[26]. Nevertheless, consumption of one-third of the prescribed amount of each of the dairy food products was estimated by 24-hour recall, a method that is widely considered as the most appropriate for measuring current diet in groups of subjects[27]. Furthermore, previous studies of this kind in pregnant Chilean women have similarly shown a daily intake of one-third of the prescribed amount for powdered milk[12].

The idea of using a combination of micronutrients either as a supplement or a fortified food is supported by several studies that showed a positive difference in mean birth weight of about 60 g in infants of mostly healthy pregnant women[8–10,28]. In the latter study, 33% of women had HIV infection and they had an increase in mean birth weight of 101 g ($P = 0.057$), a figure similar to that obtained in another study of multivitamin supplementation among HIV-1-infected women in Tanzania who

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reached 100 g ($P = 0.01$)[7]. In the latter study severe preterm birth (<34 weeks) was also significantly reduced by 39%. Nevertheless, in a controlled trial of healthy women in Mexico, a multiple micronutrient supplement was compared with iron alone and no effects were reported on birth weight[29]. A possible explanation for the absence of additional benefit when supplementing with folic acid–iron in one of the Nepalese studies[8], or with iron alone as in the Mexican study[29], may be that pregnant women in those studies specifically lacked those micronutrients and no others. Results from this new study in Chile show that the combination of multiple micronutrients and omega-3 fatty acids can still have an impact on foetal growth when folic acid and iron are universally provided. As the possible effects of either multiple micronutrients or omega-3 fatty acids were not able to be isolated from this trial, it is still necessary to undertake new research which may establish their individual and combined effects[5,30,31].

Regarding the possible mechanisms that may explain the effect of micronutrients on foetal growth, it has been proposed that multiple micronutrients added to the regular diet may lead to higher fluid retention and greater plasma volume expansion during pregnancy[1,10,32]. This higher plasma volume expansion may favourably influence foetal growth[33]. There is evidence suggesting that maternal body water near term is an important variable influencing birth weight[34].

Changes in gestation duration were not observed in any of the above-mentioned trials that used a combination of micronutrients in mostly healthy women[8–10,28]. Omega-3 fatty acids in this trial may account for the observed statistically significant longer gestation duration. It has been proposed that they may prolong gestation through possible effects on prostaglandins that play a key role in the timing of parturition[35]. However, the possible mechanisms and the effects of polyunsaturated fatty acids during pregnancy are still under considerable debate and investigation[3,36,37]. The statistically significant two days increase in gestation duration in the experimental group, although without enough power, is consistent with the higher birth weight in this group. During the last stage of gestation mean birth weight usually increases by about 200–300 g per week[38]; two days of longer gestation could partly explain the increase in birth weight in our study.

It is generally believed that prenatal factors, such as nutritional supplementation during pregnancy, are more likely to affect birth weight than birth length[39]. Our results provide new evidence for the possibility that better food quality may also enhance birth length.

One trial of vitamin and mineral supplementation found increased head circumference[8] and it has been suggested that omega-3 might also have a positive effect on this outcome[36].

The observed negative association between product M and pre-eclampsia could possibly be influenced by the presence of vitamins or omega-3 fatty acids. Although a possible role of concomitant supplementation with vitamins C and E on the prevention of pre-eclampsia was not found in a recent randomised controlled supplementation trial[40], an increased risk of pre-eclampsia associated with low plasma levels of vitamin B_{12} has been recently shown[31]. On the other hand, the possible negative association between omega-3 intake and incidence of pre-eclampsia is controversial[35].

Two recent trials in Nepal reported increased perinatal mortality in the groups of women receiving multiple micronutrients, possibly due to an increase in birth asphyxia, resulting from larger newborn size and obstructed delivery[9,41]. The increased perinatal and neonatal mortality was statistically significant when the data from both trials were combined[42]; nevertheless, the authors concluded that it is presently inappropriate to extrapolate these findings to other populations[43]. In our study, there were no increases in instrumental delivery, birth asphyxia, stillbirths or neonatal deaths. Our findings do not suggest that maternal micronutrient supplementation may increase birth asphyxia and perinatal or neonatal mortality in our population. Although we do not have data on infant mortality, in this trial there were no perinatal or neonatal deaths.

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