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Compared with Cow Milk, a Growing-Up Milk Increases Vitamin D and Iron Status in Healthy Children at 2 Years of Age: The Growing-Up Milk–Lite (GUMLi) Randomized Controlled Trial

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Abstract

Background: Iron deficiency (ID) and vitamin D deficiency (VDD) are significant pediatric health issues in New Zealand and Australia and remain prevalent micronutrient deficiencies in young children globally.

Objective: We aimed to investigate the effect of a micronutrient-fortified, reduced-energy growing-up milk (GUMLi) compared with cow milk (CM) consumed for 1 y on dietary iron and vitamin D intakes and the status of New Zealand and Australian children at 2 y of age.

Methods: The GUMLi Trial was a multicenter, double-blind, randomized controlled trial in 160 healthy 1-y-old New Zealand and Australian children conducted in 2015–2017. Participants were randomly assigned 1:1 to receive GUMLi (1.7 mg Fe/100 mL; 1.3 μg cholecalciferol/100 mL) or CM (0.02 mg Fe/100 mL; 0.06 μg cholecalciferol/100 mL) for 12 mo. Secondary outcomes, reported here, included change in dietary iron and vitamin D intakes, iron status, and 25-hydroxyvitamin D [25(OH)D] concentrations from blood samples at age 2 y. All regression models were adjusted for baseline outcome and study center.

Results: GUMLi was a large contributor to dietary intakes of iron and vitamin D after 12 mo when compared with intakes from food and CM. The adjusted mean difference between groups for serum ferritin concentrations was 17.8 μg/L (95% CI: 13.6, 22.0 μg/L; $P < 0.0001$), and for 25(OH)D it was 16.6 nmol/L (95% CI: 9.9, 23.3 nmol/L; $P < 0.0001$). After 12 mo, ID was present in 16 (24%) participants in the CM group and 5 (7%) participants in the GUMLi group ($P = 0.009$), and the prevalence of VDD in the CM group increased to 14% ($n = 10$) and decreased to 3% ($n = 2$) ($P = 0.03$) in the GUMLi group.

Conclusion: In comparison with CM, GUMLi significantly improved dietary iron and vitamin D intakes and the iron and vitamin D status of healthy children at 2 y of age. This trial was registered with the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au) as ACTRN12614000918628. *J Nutr* 2018;148:1570–1579.

Keywords: child, iron deficiency, vitamin D deficiency, nutritional status, fortified milk

Introduction

Micronutrient deficiencies are a prevalent cause of infant morbidity and mortality (1). Poor nutrition, including micronutrient undernutrition, is associated with an increased susceptibility to infection and an increased risk of repeated acute infections (1–3). Iron is a known limiting nutrient in the diets of children aged <2 y (4). Iron deficiency (ID) and vitamin D deficiency (VDD) are prevalent issues, and ID remains one of the most common nutrient deficiencies in young children worldwide (4–7).

Requirements for iron (milligrams per kilogram) during the first 2 y of a child's life are high compared with any other life stage (5), resulting in an increased vulnerability to suboptimal iron concentrations and risk of developing ID (8). ID within the first 2 y may be associated with reduced educational attainment at school (5) and poorer functioning in cognitive, affective, and motor domains at 5 y of age (9). Findings from population-based New Zealand studies showed that the prevalence of ID ranges from 14% to 29% in 6- to

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24-mo-old children in Auckland (3, 10), with ID anemia (IDA) present in 7% of infants at 12 and 18 mo of age (11). Variation in prevalence is associated with ethnicity and cultural dietary practices, but not socioeconomic status (3, 12). Nutritional risk factors include excessive consumption of cow milk (CM) (13), breastfeeding beyond 6 mo of age without receiving iron-fortified products, and increased body mass (3). Similar findings have been reported in Australia, where the incidence of ID in 6- to 24-mo-old children ranged from 3% to 25% and IDA is seen in 6% of children aged ≤36 mo of age (6, 14–16).

There are limited data describing the vitamin D status of children under the age of 2 y. Low vitamin D status and subsequent VDD cause rickets, poor growth, and increased risk of acute respiratory infections and atopic sensitization (17–20). In New Zealand, VDD was prevalent in 10% of children aged 6–23 mo (21) and in ≤78% of young children during winter (22), with variations in prevalence associated with season and ethnicity (21, 23). Additional risk factors include female sex, higher BMI, darker skin color, prolonged breastfeeding, no infant formula consumption, not taking vitamin D supplements, and living in a crowded household (20–23). Data on the prevalence of VDD in Australian children aged <2 y are scarce; however, population surveys have identified VDD in 10% of children aged 8–10 y (24, 25).

Both New Zealand and Australian Dietary Guidelines recommend offering whole CM alongside breast milk and water from 1 y of age (26, 27). CM is a rich source of energy, protein, and minerals and contributes to a high percentage of energy intake in young children (28, 29). However, the concentration and bioavailability of iron are low and thus CM can have adverse effects on iron status (8, 30). CM can be a good vehicle for fortification (31–34) and growing-up milks (GUMs), also referred to as young-child formula (YCF), are milk-based drinks that provide nutrients for which there may sometimes be marginal intake (e.g., iron and vitamin D) during the dietary transition phase of early childhood (35, 36). However, their use to improve general nutritional status and health outcomes in young children requires further evaluation, and to our knowledge, the role of GUMs in the whole diets of young New Zealand and Australian children has not yet been explored.

The aim of this secondary analysis was to investigate the effect of a micronutrient-fortified, reduced-protein GUM (or GUM-Lite; GUMLi), given for 52 wk, on iron status and 25-hydroxyvitamin D [25(OH)D] concentrations at 2 y of age compared with standard CM. The effects of the intervention on dietary iron and vitamin intake and the prevalence of ID, IDA, and VDD were also determined.

Methods

Study design. The GUMLi Trial was a multicenter, double-blind, randomized controlled trial performed in urban central and greater areas of Auckland, New Zealand (n = 108), and Brisbane, Australia (n = 52), from January 2015 to January 2017. Ethical approval was obtained from the Northern B Health and Disability Ethics Committee of the Ministry of Health, New Zealand (14/NTB/152), and the University of Queensland Medical Research Ethics Committee, Brisbane, Australia (2014001318). The trial has been registered with the Australian New Zealand Clinical Trials Registry (ACTRN12614000918628). Trial reporting was guided by CONSORT (Consolidated Standards of Reporting Trials) statements (37). Primary caregivers were required to have a sufficient level of written or spoken English to provide written informed consent on their child's behalf and to allow participation in the study.

Inclusion and exclusion criteria. Participants were 160 healthy young children aged 1 y (±2 wk). Exclusion criteria were as follows: born at <32 weeks of gestation, self-reported diagnosed illness likely to influence nutritional status or growth (e.g., chronic illness known to cause malabsorption), diagnosed developmental disability (e.g., autism, intellectual disability), taking medication likely to interfere with iron absorption, receiving iron supplementation, a baseline blood test hemoglobin concentration of <100 g/L, or a diagnosed CM allergy.

Randomization and blinding. Each participant was randomly assigned at a 1:1 ratio to 1 of 2 treatment groups: a nonfortified CM (control) or GUMLi, a reduced-energy and -protein, micronutrient-fortified (vitamin D and iron) CM-based GUM product (intervention), for a period of 52 wk. An independent statistician produced the randomization lists using computer-generated randomization sequences, stratified by study center (Auckland and Brisbane).

Intervention. Participants were requested to consume 300 mL supplied milk/d. Primary caregivers were contacted by phone on a monthly basis to discuss study-milk adherence, any potential issues or adverse events (e.g., gastrointestinal upset or taste acceptance), or use of medication, and to record the study child's recent health and daily study-milk consumption over the month. Adherence was defined as consumption of ≥300 mL study milk/d on 80% of the days within the monitored interval (i.e., the previous month).

Brief nutrient profiles of both milk products are shown in Table 1. The full nutritional profile can be found in **Supplemental Table 1.** Excessive energy and protein intakes during early childhood and the influence on later adiposity have been identified in the literature (38–42). The primary outcome was to investigate the effect of an energy- and protein-reduced GUM on body fat percentage at 2 y of age. GUMLi is lower in energy and protein than standard commercial GUM products: 55 kcal/100 mL compared with 71 kcal/100 mL and 1.7 g/100 mL compared with 2.2 g/100 mL, respectively. Nonfortified CM was used as an active control and was energy matched to GUMLi, but had a protein content of 3.1 g/100 mL (compared with 1.7 g/100 mL). GUMLi was also fortified with iron (1.7 mg/100 mL), cholecalciferol (1.2 μg/100 mL), and additional probiotics and prebiotics (synbiotics). The control product contained 0.02 mg Fe/100 mL and 0.06 μg cholecalciferol/100 mL. Both study-milk products were manufactured in a registered facility and complied with Food Standards Australia and New Zealand guidelines. They were produced, packaged, and allocated (for blinding purposes) by Danone Nutricia Research (Oceania). The milks were provided in powder form with preparation instructions for dilution with water and were supplied to participants at no cost. Parents were not given any dietary advice for their child to follow during the intervention period and continued breastfeeding was

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Supplemental Table 1 and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.
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Abbreviations used: CM, cow milk; CRP, C-reactive protein; EAR, Estimated Average Requirement; GUM, growing-up milk; GUMLi, growing-up milk–lite; ID, iron deficiency; IDA, iron deficiency anemia; MCV, mean corpuscular volume; VDD, vitamin D deficiency; YCF, young-child formula; 25(OH)D, 25-hydroxyvitamin D.

TABLE 1 Nutritional composition of CM and GUMLi per 100 mL of prepared product[1]

	Study group	
	Control (CM)	Intervention (GUMLi)
Energy, kJ	245.0	249.0
Macronutrients, g		
Protein	3.1	1.7
Carbohydrate	4.5	7.8
Total fat	3.1	1.9
Saturated fat	1.9	1.3
Total n–3 long-chain FAs (DHA+EPA+DPA), g	<0.002	0.04
Dietary fiber, g		
scGOS	0.0	1.8
lcFOS	0.0	0.2
Micronutrients		
Nonheme iron, mg	0.0	1.3
Cholecalciferol, μg	0.1	1.2

[1]Values obtained from the manufacturer (Danone) and are based on average totals from 3 batches produced for use in the GUMLi Trial. CM, cow milk; DPA, docosapentaenoic acid; GUMLi, Growing-Up Milk–Lite; lcFOS, long-chain fructo-oligosaccharides; scGOS, short-chain galacto-oligosaccharides.

encouraged and supported throughout the trial. Parents were asked to discontinue any use of infant formula at baseline and to only offer the study milk for the duration of the intervention. If their child required additional milk each day, parents were asked to offer standard CM.

Data were collected in a clinical setting at baseline (i.e., at 1 y old), study month 6, and the 12-mo final appointment (i.e., at 2 y old) and in a home visit at study months 3 and 9. Primary caregivers completed a baseline questionnaire about their and the study child's medical history, demographic characteristics, and household characteristics; the study child's day-care attendance; and sunlight exposure. An indirect measurement of exposure to UV-B radiation was made by determining the hours per day the child spent outside in the sun in the preceding 4 wk. Reported sunlight exposure has been shown to correlate with serum 25(OH)D in New Zealand (21, 43). A blood sample was collected by a trained phlebotomist at the baseline visit via 1 of 2 methods: *1*) a 5-mL venous blood sample or 2) a 3-mL capillary sample, depending on parent preference. A second blood sample was collected in an identical manner to the baseline sample collection on completion of the study (month 12) (**Supplemental Figure 1**). Dietary intake was assessed by using the Eating Assessment in Toddlers FFQ (EAT FFQ) at 5 time points (baseline and study months 3, 6, 9, and 12), and included questions on supplement use. The EAT FFQ has shown high correlations for energy and nutrients, including iron when compared with diet records (44, 45). It had not been validated for vitamin D intake, but in this study there was a good correlation for energy-adjusted vitamin D intake between the FFQ and four 24-h recalls ($r = 0.57$, $P < 0.0001$).

Laboratory analyses. Hemoglobin, mean corpuscular volume (MCV), serum ferritin, serum iron, transferrin saturation, transferrin, and C-reactive protein (CRP) were measured by LabTests (Auckland, New Zealand) and Pathology Queensland (Brisbane, Australia). Both laboratories participate in regular external quality-assurance testing. Hemoglobin and MCV were analyzed with an automated hematology analyzer, Sysmex XN-10/X-20 (Roche Diagnostics Ltd.) and Sysmex XN-3000 (Roche Diagnostics Pty. Ltd.). Serum ferritin was measured by using a Latex microparticle enhanced immunoturbidometric assay (ADVIA Chemistry XPT Systems; Siemens Healthcare Diagnostics, Inc.) in Auckland and a Unicel DXI 800 Access Immunoassay System (Beckman Coulter, Inc.) in Brisbane. CRP was measured by using a Latex enhanced immunoturbidometric assay (ADVIA Chemistry XPT Systems; Siemens Healthcare Diagnostics, Inc.) in Auckland and a Siemens Behring Nephelometer II (Siemens) in Brisbane. Serum iron was measured by using a Ferrozine assay (ADVIA Chemistry XPT Systems; Siemens Healthcare Diagnostics, Inc.) in Auckland and a Unicel DXI 800 Access Immunoassay System (Beckman Coulter, Inc.) in Brisbane. Transferrin was measured by using polyethylene glycol-enhanced immunoturbidometric assay (ADVIA Chemistry XPT Systems; Siemens Healthcare Diagnostics, Inc.) in Auckland and a Unicel DXI 800 Synchron Chemical System (Beckman Coulter, Inc.) in Brisbane. Plasma samples (0.5 mL) for 25(OH)D analysis were stored at –80°C until study completion and were measured with the use of isotope dilution LC–tandem MS in a Vitamin D External Quality Assurance Scheme–certified laboratory (Canterbury Health Laboratories, New Zealand) (46). ID was defined as abnormal values for 2 of the following variables: MCV (≤70 fL), serum ferritin (<12 μg/L), serum iron (≤5 μmol/L), or transferrin saturation (≤0.10%) (47). IDA was defined as a hemoglobin <110 g/L and abnormal values for 2 of the following variables: MCV, serum ferritin, serum iron, or transferrin saturation (47). Primary caregivers of children identified as having IDA were notified and appropriate recommendations for treatment made to the nominated family doctor by the trial physician. VDD was defined as serum 25(OH)D <50 nmol/L. The baseline and completion vitamin D samples were obtained at similar times of the year due to the 12-mo duration of the study, so adjustment for season of blood draw and seasonal variation in circulating 25(OH)D concentrations was not required within participants.

Statistical analysis. A sample size of 64 participants in each arm was required to reach 80% power at a 5% significance level (2-sided) to detect a 0.5-SD difference in percentage body fat (primary outcome) between the 2 arms at the end of the 12-mo follow-up. Eighty children were targeted in each arm to allow 20% loss to follow-up, giving a total sample size of 160 required for the trial. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc.). All of the statistical tests were 2-sided, with significance set at $P < 0.05$. Because this analysis was for secondary outcomes, missing data were not imputed in the analysis and there was no adjustment for multiple testing. Baseline study participant and parental characteristics were summarized by treatment group with the use of descriptive statistics, in which categorical variables were described as frequencies and percentages and continuous variables as means ± SDs. No formal statistical tests were performed at baseline, because any imbalances that may be observed between treatment groups must have occurred by chance due to randomization (37).

This study of secondary outcomes included dietary iron and vitamin D intakes, iron status, 25(OH)D concentrations, and the prevalence of ID, IDA, and VDD at 2 y of age. As a secondary analysis of the main trial, all outcomes were analyzed in a modified intention-to-treat population of participants with normal CRP concentrations (<5 mg/L) at baseline and at month 12. Those with CRP concentrations ≥5 mg/L were requested to have repeat studies performed 1 mo later. The repeat iron studies and hemoglobin values were used if CRP concentrations were <5 mg/L. If CRP concentrations remained ≥5 mg/L, participants were excluded from the analysis. The use of CRP as an indicator for repeat measurements and exclusion of values ≥5 mg/L were to minimize the impact of values for serum ferritin (an acute-phase protein) becoming distorted in the presence of infection, even when iron stores are low. IDA at baseline was defined as hemoglobin <100 g/L and abnormal values for 2 of the following parameters: MCV, serum ferritin, serum iron, or transferrin saturation) (47). IDA was present in 2 participants who were excluded. To screen for implausible estimated energy intakes, the method of Huang et al. (48) was used. Tukey's fences methods of IQR were calculated as a lower cutoff [quartile 1 – (1.5 × IQR)] and an upper cutoff [quartile 3 + (1.5 × IQR)]. All remaining participants were analyzed in the groups to which they were allocated, regardless of whether or not they adhered to the intervention protocol. The Estimated Average Requirement (EAR) cutoff method was used (49) to determine the prevalence estimate of intakes below the EAR for iron and the Adequate Intake for vitamin D (50). ANCOVA regression models were used to investigate the effect of the study milks on markers of iron status and 25(OH)D concentrations after 12 mo of the intervention, adjusting for baseline outcome and study location. ID, IDA, and VDD were summarized as frequencies and percentages and compared between the 2 groups at 12 mo of the intervention using chi-square test or

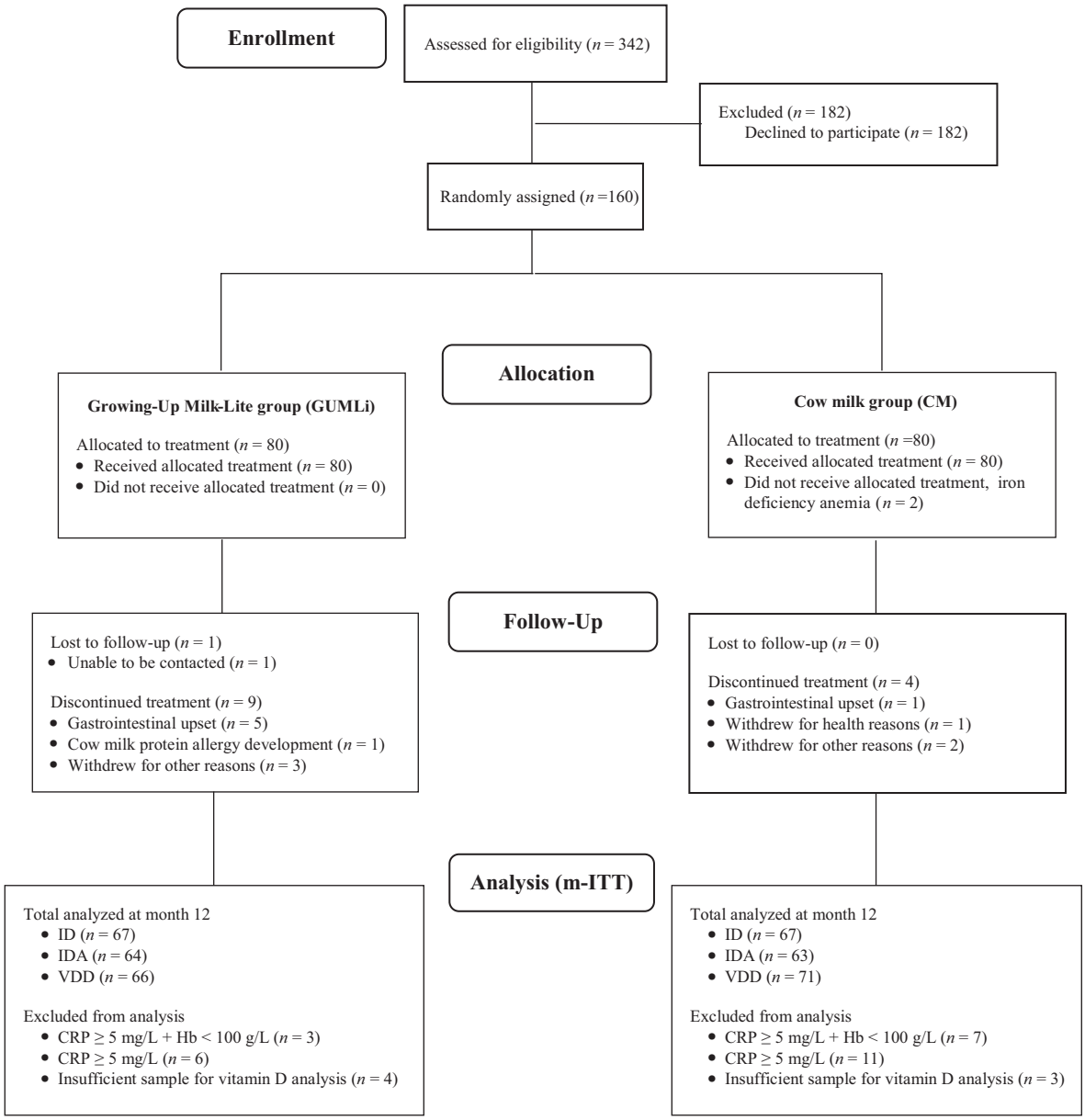

FIGURE 1 CONSORT flow diagram showing participant flow and study analysis. Children with elevated high-sensitivity CRP concentrations (≥5 mg/L) were excluded from the analyses for iron status in an attempt to control for falsely elevated or normal ferritin concentrations in the presence of infection. CONSORT, CONsolidated Standards Of Reporting Trials; CRP, C-reactive protein; GUMLi, Growing-Up Milk–Lite; Hb, hemoglobin; ID, iron deficiency; IDA, iron deficiency anemia; m-ITT, modified intention-to-treat; VDD, vitamin D deficiency.

Fisher's exact test with small cell counts <5. Logistic regression was also conducted on binary outcomes with the same covariate adjustment. Model-adjusted estimates on the group difference were reported with associated 95% CIs and *P* values.

Results

Population baseline characteristics. A total of 160 children aged 1 y (±2 wk) met the inclusion criteria and were randomly assigned to the GUMLi trial (Figure 1). Average adherence was 89% over the 12-mo period. Five (3%) participants, 2 CM and 3 GUMLi, were defined as nonadherent throughout the 12-mo duration of the study. Reasons for participant withdrawal included minor adverse events such as gastrointestinal complaints or problems with taste acceptance, usually within the first weeks of the study. No serious adverse events were recorded. After screening for implausible energy intakes, 3 participants (2 CM and 1 GUMLi) were excluded from the available nutrient data at baseline (n = 157), and after 12 mo of the intervention 5 participants (1 CM and 4 GUMLi) were excluded from the available nutrient data (n = 136). Table 2 shows the baseline maternal and child characteristics of the 2 treatment groups, respectively. The table shows that >50% of children were born at term, >50% were breastfed at baseline, a greater proportion of children enrolled in spring and winter months, and >70% of mothers had received tertiary education. There were no differences between groups at baseline, apart from the use of protective clothing or sunscreen, which was greater in the intervention group than in the control group (11% compared with 3%, respectively). At baseline, children in the GUMLi group had higher intakes of total milk per day than the CM group (567 compared with 489 mL/d, respectively); however, a

TABLE 2 At-birth and baseline characteristics of participants and parents[1]

Characteristic	Study group	
	Control ($n = 80$)	Intervention ($n = 80$)
Location		
Auckland	56 (70)	52 (65)
Brisbane	24 (30)	28 (35)
Seasons (months)		
Spring (September, October, November)	24 (30)	19 (24)
Summer (December, January, February)	13 (16)	9 (11)
Autumn (March, April, May)	19 (24)	17 (21)
Winter (June, July, August)	24 (30)	35 (44)
Gestation (completed weeks of gestation)		
Term (≥37 wk)	73 (91)	78 (98)
Preterm (<37 wk)	7 (9)	2 (2)
Child's sex		
Male	45 (56)	40 (50)
Female	35 (44)	40 (50)
Child attended day care		
No	39 (49)	41 (51)
Yes	41 (51)	39 (49)
Breastfed at baseline		
Yes	46 (58)	47 (60)
No	34 (43)	31 (40)
Missing	0	2
Child uses sunscreen/protective clothing		
No	9 (11)	2 (3)
Yes	71 (89)	78 (98)
More than 1 h spent outside/d		
No	30 (30)	22 (28)
Yes	55 (70)	57 (72)
Child uses supplements containing iron		
No	79 (99)	78 (98)
Yes	1 (1)	2 (2)
Child uses supplements containing vitamin D		
No	78 (98)	78 (98)
Yes	2 (2)	2 (2)
Mother's ethnicity		
Māori	7 (9)	8 (10)
Pacific	3 (4)	0 (0)
Asian	5 (6)	3 (4)
European	51 (64)	56 (70)
Other	14 (18)	13 (16)
Mother's highest level of education		
No school qualifications	0 (0)	0(0)
Primary	0 (0)	2 (3)
Secondary	14 (18)	12 (15)
Tertiary	57 (71)	62 (78)
Other	9 (11)	4 (5)
Mother's employment status		
Full-time caregiver	22 (28)	23 (29)
Full-time paid employment	24 (30)	16 (20)
Part-time paid employment	30 (38)	28 (35)
Receiving a benefit	1 (1)	2 (3)
Unemployed, not receiving a benefit	1 (1)	4 (5)
Other	1 (1)	7 (9)
Prefer not to answer	1 (1)	0 (0)
Milk intake during previous month		
Cow milk	55 (69)	55 (69)
Fortified milk[3]	63 (79)	59 (74)
Total amount consumed,[4] mL/d	489 ± 315	567 ± 487

[1] Values are *n* (%) unless otherwise indicated.
[2] Chi-square test or Fisher's exact test was used to determine the difference between groups for categorical variables; the Kruskal-Wallis test was used to compare the medians between groups for continuous variables.
[3] Includes infant formula, follow-on formula, or growing-up milk only.
[4] Values are means ± SDs.

greater number of children in the CM group consumed fortified milk (i.e., infant formula, follow-on formula, or GUM) than those in the GUMLi group (79% compared with 74%).

Dietary intake of iron and vitamin D and the contribution of GUM to iron and vitamin D intakes. Primary caregivers were not provided with any dietary advice during the trial period. At baseline, mean total iron (milligrams per day) and vitamin D intakes (micrograms per day) were comparable: 9.8 compared with 10.0 mg Fe/d for the GUMLi and CM groups, respectively, and 6.6 compared with 6.4 μg vitamin D/d for the CM and GUMLi groups, respectively (Table 3). GUMLi provided 1.7 g Fe/100 mL (5.1 g/300 mL per protocol) compared with CM, which provided 0.0 g Fe/100 mL (0.1 g/300 mL per protocol). After 12 mo of the intervention, GUMLi provided 40 times (40% of total iron intake) more iron than the CM (1% of total iron intake) (Table 3). The adjusted mean difference in total iron intakes (food + milk) between the intervention and control at 12 mo was 5.1 mg/d (95% CI: 4.1, 6.1 mg/d; $P < 0.0001$) and the adjusted mean difference in iron intake from fortified milks only was 5.0 mg/d (95% CI: 4.6, 5.5 mg/d; $P < 0.0001$).

With the use of the EAR cutoff method (51, 52), 13% ($n = 10$) of participants in the CM and 11% ($n = 9$) of participants in the GUMLi group had intakes below the EAR of 4 mg Fe/d at baseline (50). After the 12-mo intervention, 24% ($n = 17$) of participants in the CM group had intakes below the EAR and 1.5% ($n = 1$) had intakes below the EAR in the GUMLi group. No participants exceeded the upper limit of 20 mg/d for children aged 1–3 y (50) after 12 mo of the intervention.

After 12 mo of the intervention, the adjusted mean difference in total vitamin D intakes was 3.3 μg/d (95% CI: 2.5, 4.2 μg/d; $P < 0.0001$). The adjusted mean difference in vitamin D intake from standard CM between the GUMLi and CM groups after 12 mo was –0.3 μg/d (95% CI: –0.6, –0.1 μg/d; $P = 0.006$) and vitamin D intake from fortified milk only was 3.8 μg/d (95% CI: 3.5, 4.2 μg/d; $P < 0.0001$).

Prevalence of ID and IDA in infants fed GUMLi compared with CM. The adjusted mean difference in serum ferritin concentrations between the GUMLi and CM groups was 17.8 μg/L (95% CI: 13.6, 22.0 μg/L; $P < 0.0001$). The estimated mean ± SD change from baseline in serum ferritin concentration was –21.0 ± 24.0 μg/L in the CM group and 2.1 ± 17.9 μg/L in the GUMLi intervention group (Table 4). Table 5 shows the

TABLE 3 Daily iron and vitamin D intakes at baseline and after 12 mo of the intervention from food, milk, and fortified milks of children in the GUMLi trial[1]

	Control		Intervention			
	n	Values	*n*	Values	Adjusted difference[2] (95% CI)	*P*
Iron, mg/d						
Total						
Baseline	77	9.8 ± 3.9	80	10.0 ± 3.6		
12 mo	70	7.5 ± 2.8	66	12.7 ± 3.2	5.2 (4.2, 6.2)	<0.0001
Food only						
Baseline	77	6.0 ± 2.6	80	6.5 ± 2.8		
12 mo	70	7.3 ± 2.7	66	7.6 ± 2.7	0.3 (–0.6, 1.2)	0.56
Total unfortified milk[3]						
Baseline	77	0.0 ± 0.0	80	0.0 ± 0.1		
12 mo	70	0.1 ± 0.2	66	0.1 ± 0.1	–0.1 (–0.1, 0.0)	0.07
Fortified milk						
Baseline[4]	77	3.7 ± 3.2	80	3.5 ± 3.1		
12 mo[5]	70	0.0 ± 0.0	66	5.0 ± 2.0	5.0 (4.5, 5.5)	<0.0001
Vitamin D, μg/d						
Total						
Baseline	77	6.6 ± 3.8	80	6.4 ± 3.6		
12 mo	70	4.4 ± 2.5	66	7.8 ± 2.6	3.4 (2.6, 4.3)	<0.0001
Food only						
Baseline	77	1.9 ± 1.2	80	1.8 ± 1.4		
12 mo	70	3.3 ± 2.5	66	3.3 ± 1.8	–0.0 (–0.7, 0.7)	0.95
Total unfortified milk[3]						
Baseline	77	0.4 ± 0.8	80	0.5 ± 0.9		
12 mo	70	1.0 ± 0.7	66	0.7 ± 0.6	–0.3 (–0.5, –0.1)	0.01
Fortified milk						
Baseline[4]	77	4.3 ± 3.7	80	4.1 ± 3.7		
12 mo[5]	70	0.0 ± 0.0	66	3.8 ± 1.5	3.8 (3.5, 4.2)	<0.0001

[1]Values are means ± SDs unless otherwise indicated. Sums may be less than the total due to missing values or exclusions due to implausible energy intakes. GUMLi, Growing-Up Milk–Lite.
[2]An ANCOVA model was used to test the difference between the 2 groups, adjusting for baseline outcome and study location.
[3]Includes flavored milk, standard cow milk, and low-fat cow milk.
[4]Includes infant formula, follow-on formula, or growing-up milk only.
[5]GUMLi intervention milk only.

prevalence of ID and IDA at baseline and after the intervention. At baseline, 14 (21%) of the CM participants and 8 (11%) of the GUMLi participants had ID. After 12 mo of the intervention, ID was present in 16 (24%) participants in the CM group and 5 (7%) in the GUMLi group (chi-square test, $P = 0.009$). After adjusting for baseline prevalence of ID and study location, there was a tendency for the odds of ID after the intervention to be lower in the GUMLi group than in the CM group (adjusted OR: 0.32; 95% CI 0.09, 1.09; $P = 0.068$).

The adjusted mean difference in hemoglobin concentrations was 2.8 g/L (95% CI: 0.1, 5.5 g/L; $P = 0.045$) after 12 mo of the intervention. At baseline, 2 participants were identified as having IDA and were excluded (Table 5). After 12 mo of the intervention, IDA was present in 4 participants (6%) in the CM group and in 1 participant (2%) in the GUMLi group (chi-square test, $P = 0.208$). However, there was no evidence that the odds of IDA were lower in the GUMLi group than in the CM group after adjusting for study location (adjusted OR: 0.23; 95% CI: 0.02, 2.09; $P = 0.190$).

Vitamin D status and prevalence of deficiency. The adjusted mean difference in 25(OH)D concentrations was 16.6 nmol/L (95% CI: 9.9, 23.3 nmol/L; $P < 0.0001$) after 12 mo of the intervention (Table 4). At baseline, 5 (6%) of the CM participants and 5 (7%) of the GUMLi participants had VDD. After 12 mo of the intervention, VDD was present in 10 (14%) participants in the CM group and 2 (3%) participants in the GUMLi group (chi-square test, $P = 0.02$). After adjusting for baseline prevalence of VDD and study location, the odds of VDD after the intervention were lower in the GUMLi group than in the CM group (adjusted OR: 0.16; 95% CI: 0.03, 0.82; $P = 0.028$).

Discussion

We showed that when consumed as part of a whole diet for 12 mo, GUMLi preserves iron stores and significantly reduces the prevalence of ID ($P = 0.009$) and VDD ($P = 0.02$) compared with CM consumption at 2 y of age. The effects of the intervention were also seen, with GUMLi significantly improving total iron and vitamin D intakes ($P < 0.0001$). We had high levels of protocol adherence over the 12-mo intervention (89%), indicating that the consumption of ≥300 mL GUMLi/d was an acceptable method of delivering a fortified food. Our findings show that GUMLi could be used as a strategy to increase dietary iron and vitamin D intakes and decrease the occurrence of ID and VDD in young New Zealand and Australian children.

Iron and vitamin D status. The development of ID was significantly less likely ($P = 0.009$) after 12 mo of the intervention in participants in the GUMLi group (7%) compared with

TABLE 4 Iron and vitamin D status before and after the GUMLi trial intervention[1]

	Control		Intervention			
	n	Values	*n*	Values	Adjusted difference[2] (95% CI)	*P*
Hemoglobin, g/L						
Baseline	67	117.9 ± 8.2	69	116.9 ± 7.4		
12 mo	63	120.3 ± 9.4	64	122.8 ± 6.6	2.8 (0.1, 5.5)	0.05
Serum ferritin, μg/L						
Baseline	67	38.7 ± 37.0	69	30.5 ± 20.4		
12 mo	67	14.5 ± 8.5	67	30.9 ± 14.3	17.8 (13.6, 22.0)	<0.0001
Serum iron, μmol/L						
Baseline	67	8.8 ± 4.9	68	8.9 ± 4.1		
12 mo	67	10.8 ± 6.4	67	11.5 ± 4.9	0.4 (–1.7, 2.5)	0.69
Serum transferrin, g/L						
Baseline	67	2.7 ± 0.4	69	2.7 ± 0.3		
12 mo	67	3.1 ± 0.6	67	2.7 ± 0.4	–0.5 (–0.6, –0.3)	<0.0001
Transferrin saturation, %						
Baseline	67	4.5 ± 7.0	68	5.3 ± 8.2		
12 mo	66	4.8 ± 7.5	67	6.3 ± 9.6	1.8 (0.1, 3.6)	0.04
MCV, fL						
Baseline	66	74.8 ± 3.7	69	75.2 ± 3.7		
12 mo	63	74.0 ± 4.6	64	76.3 ± 3.6	2.5 (1.4, 3.6)	<0.0001
Serum 25-hydroxyvitamin D, nmol/L						
Baseline	77	85.9 ± 23.4	76	90.4 ± 28.8		
12 mo	71	74.9 ± 23.5	66	92.0 ± 25.5	16.6 (9.9, 23.3)	<0.0001

[1] Values are means ± SDs unless otherwise indicated. Iron analyses were performed in a modified intention-to-treat sample in which children with an elevated CRP (>5 g/L) were excluded to prevent false elevation of normal ferritin concentrations as seen in the presence of infection. Sums may be less than the total due to missing values or exclusion in the modified intention-to-treat model. CRP, C-reactive protein; GUMLi, Growing-Up Milk–Lite; MCV, mean corpuscular volume.
[2] An ANCOVA model was used to test the difference between the 2 groups, adjusting for baseline outcome and study location.

those in the CM group (24%). In New Zealand, a 20-wk randomized controlled trial showed that the consumption of iron-fortified milk increased iron stores in healthy, nonanemic toddlers compared with unfortified CM, whereas increased consumption of red meat prevented a decrease in these stores (53, 54). Only iron-fortified milk was likely to ensure an increase in iron stores and be protective against ID (3, 53–55), with a clinically important difference of 42% detected in mean serum ferritin concentrations between the fortified-milk group and the control after 20 wk (53). We were able to detect a larger, clinically meaningful difference of 47% in serum ferritin concentrations between the CM and GUMLi groups at the end of our 52-wk intervention.

Micronutrient deficiencies most often occur concurrently; therefore, there is potential that multimicronutrient fortification, as seen in GUMLi, may result in more positive effects on markers such as iron and 25(OH)D. This was shown in a systematic literature review in older children (56) and more recently in a multicenter European GUM randomized controlled trial, in which it was suggested that combined fortification may

TABLE 5 Iron and vitamin D deficiency before and after the GUMLi trial intervention[1]

	Control, *n*/total *n* (%)	Intervention, *n*/total *n* (%)	OR[2] (95% CI)	*P*
Iron deficiency[3]				
Baseline	14/67 (21)	8/71 (11)		
12 mo	16/67 (24)	5/67 (7)	0.32 (0.09, 1.09)	0.068
Iron deficiency anemia				
Baseline[4]	0/67 (0)	0/69 (0)		
12 mo[5]	4/63 (6)	1/64 (2)	0.23 (0.02, 2.09)	0.190
Vitamin D deficiency[6]				
Baseline	5/77 (6)	5/76 (7)		
12 mo	10/71 (14)	2/66 (3)	0.16 (0.03, 0.82)	0.028

[1] CRP, C-reactive protein; GUMLi, Growing-Up Milk–Lite.
[2] Logistic regression analysis was used to test any differences between the 2 study groups, adjusting for baseline outcome (if available) and study location, $P < 0.05$ (reference group: control).
[3] Iron deficiency was defined as abnormal values for 2 out of the following variables without elevated CRP: mean corpuscular volume, serum ferritin, serum iron, transferrin saturation.
[4] Iron deficiency anemia at baseline was defined as iron deficiency combined with a hemoglobin concentration <100 g/L.
[5] Iron deficiency anemia after 12 mo of the intervention was defined as iron deficiency combined with a hemoglobin concentration <110 g/L.
[6] Vitamin D deficiency was defined as serum 25-hydroxyvitamin D concentrations <50 nmol/L.

have had a synergistic effect on iron and vitamin D status (57). In a meta-analysis by Eichler et al. (58), multimicronutrient fortification (i.e., iron and vitamin D) resulted in a mean increase in hemoglobin of 0.87 g/L (95% CI: 0.57, 1.16 g/L; 8 studies) compared with single-nutrient (i.e., iron) fortification, with a mean increase in hemoglobin of 0.62 g/L (95% CI: 0.34, 0.89 g/L; 13 studies), and with a reduction in the prevalence of IDA of 57% (RR: 0.43; 95% CI: 0.26, 0.71). Ramakrishnan et al. (59) also reported significant mean changes in hemoglobin concentrations with an effect size of 1.49 g/L (95% CI: 0.46, 2.51 g/L). We observed a larger impact on hemoglobin with an effect size of 2.8 g/L (95% CI: 0.1, 5.5 g/L; $P = 0.045$).

In a study in Western European children aged 1–3 y, a GUM with 1.2 mg Fe/100 mL resulted in a mean ± SD increase in serum ferritin of 1.7 ± 2.4 μg/L (57). The iron concentration in the current GUMLi trial was larger at 1.7 mg/100 mL, which contributed to an increase in mean ± SD serum ferritin of 2.1 ± 18.0 μg/L compared with a decrease of 21.0 ± 24.0 μg/L in the CM group, with an effect size of 17.8 μg/L (95% CI: 13.6, 22.0 μg/L; $P < 0.0001$). Our results suggest that GUMLi could play a role in preventing the development of age-related decline in iron stores, and a resultant increase in ID, in New Zealand and Australian children. A recent position paper by the European Society for Pediatric Gastroenterology, Heptology, and Nutrition Committee on Nutrition concluded that the routine use of YCF in children aged 1–3 y is not necessary based on current evidence, but that YCF could be used as one strategy to increase iron and vitamin D intakes compared with CM within this age range (60). The authors also concluded that further studies are needed to investigate the role of YCF in the diets of young children.

Dietary iron and vitamin D intakes. Data on the nutrient status of children aged <2 y are limited. The transition toward family-based foods leads to a decrease in the reliance on iron-fortified foods and an increase in foods that are poorer sources of iron (9, 37). CM consumption in young children is strongly associated with reduced iron stores and an increased risk of developing ID (8, 61). The risk of inadequate iron intakes in young children has been reported in New Zealand (10, 62), Australia (63), and globally (4, 29, 64–66). In a recent systematic review by Eussen et al. (67), inadequate intakes were observed in ≤60% of European children aged 12–36 mo, and a high prevalence of ID was observed. In the present study, we observed inadequate intakes in 24% of the CM group and 1.5% of the GUMLi group after 12 mo of the intervention. Iron intakes were higher than previously seen in New Zealand, in which the mean ± SD intake in children aged ≥12 mo was 5 ± 2.5 mg/d (62), with 15% at risk of inadequate intakes. In the Melbourne Infant Feeding, Activity and Nutrition Trial (InFANT) program, 18.6% of children aged >12 mo had inadequate iron intakes, with a mean ± SD iron intake of 6.6 ± 2.4 mg/d (63). In both studies, dietary intake was measured via weighed food records or 24-h recalls rather than an FFQ.

Diet is a poor source of vitamin D for New Zealand and Australian children, and VDD is prevalent during early childhood (3). There is a lack of policy surrounding vitamin D supplementation in infants and children and, as such, only 2% of children in each intervention arm were taking a vitamin D supplement at baseline. Mean baseline 25(OH)D concentrations were 85.9 and 90.4 nmol/L in the CM and GUMLi groups, respectively, which is higher than the previously seen mean concentration of 52 nmol/L in 12- to 22-mo-old New Zealand children (23). This may be due to a high intake of infant formula at baseline in both groups before the intervention allocation (358 mL/d in both groups). Population surveys have shown that 40–57% of newborns in New Zealand (68) and Australia (69) have 25(OH)D concentrations <50 nmol/L, with variations across seasons and ethnicity, and a persistent high prevalence of low concentrations in the first 2 y of life (70). At baseline, 6% and 7% of children in the CM and GUMLi groups, respectively, had VDD. Improvement in vitamin D status was seen in the GUMLi group, resulting in a decrease in VDD to 3% in the GUMLi group compared with an increase to 14% in the CM group. GUMLi remained protective during winter months, with an estimated mean difference in 25(OH)D of 19.1 nmol/L (95% CI: 13.0, 25.3 nmol/L; $P < 0.0001$) after 12 mo of the intervention. Daily consumption of a vitamin D–fortified GUM (2.85 μg/100 mL, double that of GUMLi) in the Kindermilch Growing-Up Milk Trial also prevented a decrease in 25(OH)D concentrations during winter months (71). This suggests that achieving a high 25(OH)D concentration during summer and spring months is not sufficient to maintain 25(OH)D concentrations >50 nmol/L all year, and that increasing the availability of vitamin D through fortified milk is independently protective against VDD (23).

Strengths and limitations. Strengths of our study include a high retention rate (88%) and good compliance (89%). Defining IDA as an exclusion criterion and removal of CRP values ≥5 mg/L meant that the baseline data were clean and a true change in the prevalence of ID and IDA could be established. The use of a cutoff hemoglobin value of 100 g/L (fifth percentile of distribution) at baseline compared with the more common value of 110 g/L (10th percentile) allowed us to classify IDA as deficiency at a stage when iron stores have been depleted to the point of decreased erythropoiesis (72). The study duration of 1 y allowed participants to enter and complete the study within the same season, avoiding the need to account for seasonal variation within participants. The exclusion of CRP values ≥5 mg/L reduced the risk of serum ferritin values being distorted in the presence of infection; however, markers of infection such as α1-glycoprotein, or soluble transferrin receptor as a marker of iron status because it is less affected by inflammation, could be considered (73). Further development of the consistency, taste, and lower gastrointestinal impact of the GUMLi intervention milk may result in greater acceptance and reduce participant withdrawal. Medically diagnosed CM allergies were an exclusion criterion; therefore, our results cannot be generalized to populations in whom there are high levels of milk allergy or lactose intolerance.

In conclusion, this study provides evidence that the delivery of micronutrients such as iron and vitamin D, through a well-accepted food such as fortified milk (i.e., GUMLi), is effective in preserving iron status, improving vitamin D status, and increasing total iron and vitamin D intakes in children aged 1–2 y in New Zealand and Australia. Young children fed unfortified CM throughout childhood may experience difficulties in maintaining good iron status, placing them at risk of ID. The consumption of GUMLi as part of a whole diet improves total iron and vitamin D intakes and reduces the prevalence of ID and VDD.

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conducted the study; ALL and CRW: wrote the manuscript; YJ and RXC: conducted the statistical analyses of the data; TAW and A-LMH: served as advisors to the core trial; and all authors: contributed to and read and approved the final manuscript.

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