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A micronutrient-fortified young-child formula improves the iron and vitamin D status of healthy young European children: a randomized, double-blind controlled trial[1]

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ABSTRACT

Background: Iron deficiency (ID) and vitamin D deficiency (VDD) are common among young European children because of low dietary intakes and low compliance to vitamin D supplementation policies. Milk is a common drink for young European children. Studies evaluating the effect of milk fortification on iron and vitamin D status in these children are scarce.

Objective: We aimed to investigate the effect of a micronutrient-fortified young-child formula (YCF) on the iron and vitamin D status of young European children.

Design: In this randomized, double-blind controlled trial, healthy German, Dutch, and English children aged 1–3 y were allocated to receive either YCF (1.2 mg Fe/100 mL; 1.7 μg vitamin D/100 mL) or nonfortified cow milk (CM) (0.02 mg Fe/100 mL; no vitamin D) for 20 wk. Blood samples were taken before and after the intervention. The primary and secondary outcomes were change from baseline in serum ferritin (SF) and 25-hydroxyvitamin D [25(OH)D], respectively. ID was defined as SF <12 μg/L in the absence of infection (high-sensitivity C-reactive protein <10 mg/L) and VDD as 25(OH)D <50 nmol/L. Statistical adjustments were made in intention-to-treat analyses for sex, country, age, baseline micronutrient status, and micronutrient intake from food and supplements (and sun exposure in the case of vitamin D outcomes).

Results: The study sample consisted of 318 predominantly Caucasian ($\sim$95%) children. The difference in the SF and 25(OH)D change between the treatment groups was 6.6 μg/L (95% CI: 1.4, 11.7 μg/L; $P = 0.013$) and 16.4 nmol/L (95% CI: 9.5, 21.4 nmol/L; $P < 0.001$), respectively. The probability of ID (OR 0.42; 95% CI:0.18, 0.95; $P = 0.036$) and VDD (OR 0.22; 95% CI: 0.01, 0.51; $P < 0.001$) after the intervention was lower in the YCF group than in the CM group.

Conclusion: Micronutrient-fortified YCF use for 20 wk preserves iron status and improves vitamin D status in healthy young children in Western Europe. This trial was registered at www.trialregister.nl as NTR3609. *Am J Clin Nutr* doi: 10.3945/ajcn.116.136143.

Keywords: iron deficiency, vitamin D deficiency, cow milk, young-child formula, vitamin D supplements, micronutrient fortification, young children

INTRODUCTION

Micronutrient deficiency is a major public health problem that even in industrialized countries contributes to the global burden of disease. Iron deficiency (ID)[7] and vitamin D deficiency (VDD) are among the most common micronutrient deficiencies in young children worldwide (1). ID can lead to iron deficiency anemia (IDA) (2), and both of these conditions are associated with impaired neurodevelopment (3–6). It has been suggested that vitamin D has an important role in immune system functioning and in preventing cancers, whereas VDD can lead to rickets (7, 8).

Despite national nutritional recommendations, the iron and vitamin D intake of young children in Europe has been shown to often be insufficient in preventing ID and VDD (9–13). Furthermore, although the use of vitamin D supplements is associated with a lower prevalence of VDD, compliance seems to be low (7, 9, 10). To increase compliance, fortification of commonly used food products has been suggested. Fortification produces a more gradual increase in serum micronutrient concentration; in addition, if consumed on a regular and frequent basis, fortified products will maintain body stores of nutrients more efficiently and effectively than intermittent supplements (1).

Several international trials have shown beneficial effects of food fortification (e.g., milk, bread, and margarine) on iron (14–23) and vitamin D (24–27) status in children. Milk is a

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[7] Abbreviations used: AE, adverse event; CM, cow milk; hsCRP, high-sensitivity C-reactive protein; ID, iron deficiency; IDA, iron deficiency anemia; ITT, intention to treat; PP, per protocol; SF, serum ferritin; VDD, vitamin D deficiency; YCF, young-child formula; 25(OH)D, 25-hydroxyvitamin D.

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popular source for delivering fortification because of its wide availability and acceptance. However, randomized controlled trials investigating the effect of this strategy in young European children are scarce. Existing studies differ in fortification dosage and outcome parameters, which hampers the comparison of results (14, 15, 17, 23, 25). Moreover, the influence of an infection [e.g., on serum ferritin (SF)] or the season (on vitamin D status) on outcome variables is not always taken into account.

The primary objective of this study (NTR3609) was to investigate the effect of a micronutrient-fortified young-child formula (YCF) given for 20 wk on ferritin concentrations of healthy children aged 12–36 mo living in Western Europe compared with the use of nonfortified cow milk (CM). Secondary objectives were to establish the effect of the intervention on the prevalence of ID and IDA, serum 25-hydroxyvitamin D [25(OH)D] concentrations, and the prevalence of VDD.

METHODS

This randomized, double-blind controlled trial was performed in Western Europe from 2012 October to 2014 September. The participating countries were Germany, (9 private pediatric clinics spread throughout the country), Netherlands, (Juliana Children's Hospital/Haga Teaching Hospital in The Hague, VU University Medical Center in Amsterdam, and Sophia Children's Hospital/Erasmus Medical Center in Rotterdam) and the United Kingdom (Royal National Orthopedic Hospital in London and St. Mary's Hospital in Newport, Isle of Wight). The study was approved by the medical ethical review board of all participating sites. The prevalence of and risk factors for ID and VDD at baseline have previously been published (9).

Inclusion and exclusion criteria

Children aged 12–36 mo with a stable health status (i.e., without any known chronic or recent acute disease) were eligible for this study. The children were familiar with and currently drinking milk products and were expected to have a study product intake $\geq$150 mL/d. Exclusion criteria were: being born preterm ($<$32 wk, or $<$37 wk with a birth weight $<$1800 g); known infection within the last week or infection needing medical assistance or treatment within the last 2 wk; known hemoglobinopathies; any case of anemia treated within the last 3 mo; a blood transfusion received within the last 6 mo; the presence of a relevant congenital abnormality; chromosomal disorder or severe disease (such as major congenital heart disease or Down syndrome); having a disorder requiring a special diet (such as food intolerance or food allergy or complaints such as reflux, constipation, and cramps); current use of antiregurgitation, antireflux, or laxative medication; participation in any other study involving investigational or marketed products within 2 wk before entering the study; known allergy or intolerance to components of the study products (e.g., milk powder, lactose, or fish protein); and vaccination with a live or live-attenuated vaccine received within the last 2 wk. Finally, parents had to be able to understand the local language and read and fill out questionnaires.

Study procedure

Subjects were recruited in 2 ways based on the local situation at the individual sites. In the Netherlands and the United Kingdom, parents of eligible subjects were informed about the study during a preoperative visit before an elective nonemergency surgical procedure (e.g., urologic surgeries, inguinal or umbilical hernia operations, or ear-nose-throat procedures). After written informed consent was obtained, the first blood drawn was combined with the placement of an intravenous catheter needed for administering general anesthesia. The subjects from Germany were recruited during a regular visit to their pediatrician. After taking the blood sample, the included children were randomly allocated to receive either micronutrient-fortified YCF (test product) or nonfortified CM (control product) for a period of 20 wk. A computer model was used for block randomization in which stratification was applied for country and sex. Parents (and their children), investigators, and treating physicians were blinded to product allocation by coding the cans containing the study products. Parents then answered questions about their child's demographic and socioeconomic characteristics, day care center attendance, sun exposure, and medical history. Food intake was measured by a food-frequency questionnaire that was adapted and translated from previously published dietary questionnaires (28–30). Micronutrient intake was calculated with the use of a Dutch nutrient databank (31). The results reflected the intake 1 mo before the baseline visit.

During the intervention period, parents were asked not to change their child's dietary habits, including the use of supplements. After 1, 5, and 15 wk, parents were contacted by phone to discuss study product compliance and completion of diaries. These diaries included daily study product intake, possible adverse events (AEs) and serious AEs, and the use of medication. Diaries on stool frequency and consistency were completed 7 d before the last 2 scheduled visits (weeks 10 and 20). Stool frequency was measured as the number of stools passed on each day of the 7 d, and stool consistency was measured on an ordered 5-point scale with pictures (1: watery; 2: soft, pudding-like; 3: soft-formed; 4: dry-formed; 5: dry hard pellets). Halfway through the study, parents were asked to visit the study center to collect a new study product and to discuss potential issues. After 20 wk in all 3 countries, a second venous blood sample was taken while subjects visited the hospital or clinic for the last time (**Figure 1**). During all 3 visits (baseline, 10 wk, and 20 wk), height or length and weight were measured. Body weight was measured to the nearest 0.1 kg with the use of a calibrated weighing scale. Height was measured to the nearest 0.2 cm, standing and without wearing shoes, with the use of a calibrated stadiometer. In those children who were not able to stand, length was measured lying down with the use of a length board to a precision of 0.2 cm. Weight-for-age z scores, height- or length-for-age z scores, and BMI-for-age z scores following WHO growth charts were calculated.

Test and control products: YCF and CM

The detailed nutrient profiles of both study products are shown in **Table 1**. The test product was a commercially available micronutrient-fortified YCF containing 1.2 mg Fe/100 mL and 1.7 μg vitamin D/100 mL. The control product was a nonfortified CM that contained 0.02 mg Fe/100 mL and no vitamin D. The energy concentrations of both products were comparable (46 kcal/100 mL for CM compared with 50 kcal/100 mL for YCF). Both YCF and CM were supplied in powdered form with instructions for preparing the milk by diluting the powder with

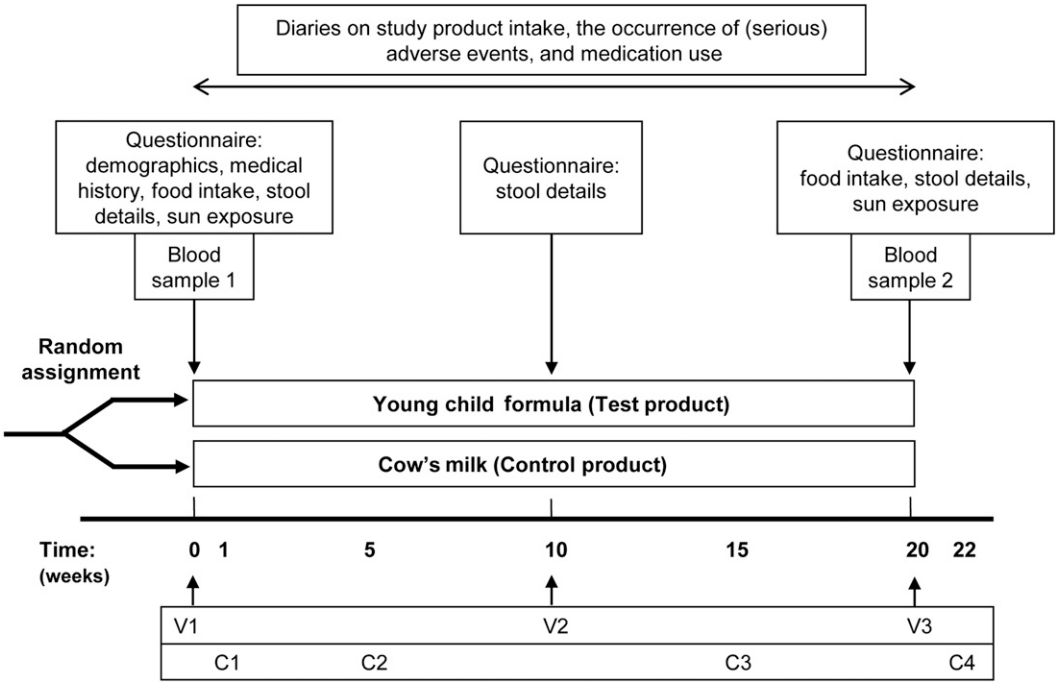

FIGURE 1 Flowchart of the study design showing the study procedure during the 20-wk intervention period and 2-wk follow-up period. C, phone contact; V, visit.

water. The study products were produced, provided, and coded (for blinding purposes) by Nutricia Cuijk (commissioned by Danone Nutricia Research).

Definitions and laboratory analyses

Blood samples were stored at Nutricia Research Analytic Science Laboratory at −80°C before being analyzed in 4 batches. Some parameters were analyzed at Reinier de Graaf Groep Laboratory. SF and serum 25(OH)D were analyzed with the use of an Abbott Architect i2000 immunology analyzer with a chemiluminesent immunoassay and chemoluminescent microparticle immunoassay, respectively.

ID was defined as SF <12 μg/L and IDA as ID combined with a hemoglobin concentration <110 g/L according to the WHO (2). Ferritin is an acute-phase protein that may increase when an infection is present, even in the presence of low iron stores. Therefore, high-sensitivity C-reactive protein (hsCRP), also an acute-phase protein, was determined in all venous blood samples, and all children with elevated hsCRP concentrations ($\geq$10 mg/L) were excluded from the ID and IDA analyses.

VDD was defined as serum 25(OH)D <50 nmol/L because this concentration is the cutoff recommended by most experts (7, 10, 32). As previously described (9), mean annual vitamin D concentrations were calculated from the single values to adjust for seasonal variations in circulating 25(OH)D concentrations with the use of the cosinor model of Sachs et al. (33).

Statistical analysis

Sample size calculations were based on the primary parameter (SF) with the use of data from Szymlek-Gay et al. (20). Assuming a difference between treatment groups in SF change (from baseline to endpoint) of 8.1 μg/L ($\pm$21 μg/L), 216 subjects (108/group) were required for a statistical power of 0.8 (α = 0.05) in a 2-sided t test. In addition, to account for stratification and dropout ($\sim$25%), 288 subjects were anticipated to be required for inclusion in the study.

Statistical analyses, described in a statistical analysis plan that was finalized before unblinding of the study, were performed with the use of SPSS version 21.0 (IBM). As a first step, the distribution of variables was assessed with the use of histograms and quantile-quantile plots. Categorical variables were then summarized by frequency and percentage distributions, and normally distributed continuous variables were summarized by means and SDs. Nonnormally distributed continuous variables were expressed as medians (IQRs) (quantiles 1 and 3).

The basic principle of our analyses was to analyze data on an intention-to-treat (ITT) basis, in which all children for whom there was information were analyzed in the groups to which they were originally allocated, irrespective of whether they actually followed the treatment regimen. Vitamin D status and hemoglobin concentrations were analyzed in this ITT study sample. Analyses regarding iron status (including IDA) were then performed in the modified ITT study sample. This sample included all subjects from the ITT study sample in whom normal hsCRP concentrations ($<$10 mg/L) were measured at both baseline and at the end of the study.

The effect of the study products on SF and serum 25(OH)D concentration was investigated with the use of linear regression analyses, whereas its effect on the prevalence of both micronutrient deficiencies was determined with the use of logistic regression analyses. In principle, these analyses were performed while adjusting for sex and country (stratification factors), age, micronutrient status at baseline, and iron or vitamin D intake (from food and supplements) at baseline. In the case of vitamin D analyses, we also adjusted for sun exposure of $\geq$1 h/d.

TABLE 1
CM and YCF content per 100 mL of prepared product[1]

	CM	YCF
Macronutrients, g		
Proteins	3.5	1.1
Carbohydrates	5.2	6.6
Fats	1.7	1.9
Fibers	0.0	0.8
Micronutrients		
Sodium, mg	40.0	20.0
Potassium, mg	174.0	56.0
Chloride, mg	101.0	31.0
Calcium, mg	127.0	110.0
Phosphorus, mg	100.0	67.0
Magnesium, mg	12.0	10.0
Nonheme iron, mg	0.02	1.2
Zinc, mg	0.40	0.90
Copper, μg	2.4	59.0
Manganese, μg	0.91	16.0
Selenium, μg	0.90	2.3
Iodine, μg	9.8	17.0
Vitamin A, μg REs	13.0	65.0
Vitamin D_3, μg	0.0	1.7
α-Tocopherol (vitamin E), mg	0.0	1.3
Vitamin K, μg	0.0	5.0
Thiamin (vitamin B-1), μg	28.0	70.0
Riboflavin (vitamin B-2), μg	142.0	87.0
Vitamin B-6, μg	30.0	60.0
Folic acid, μg	1.6	18.0
Vitamin B-12, μg	0.24	0.13
Biotin, μg	2.0	1.7
Vitamin C, mg	0.55	14.0

[1] CM, cow milk; RE, retinol equivalent; YCF, young-child formula.

Finally, all previously mentioned analyses, including adjustments for the predefined variables, were also performed in the 2 per-protocol (PP) samples. These samples consisted of subjects from the ITT and the modified ITT sample that demonstrated good compliance with instructions for consuming the assigned study product. Good compliance was defined as consuming ≥151 mL study product/d for ≥80% of the days within the last 28 d of study product intake. All CIs are 2-sided with a confidence level of 95%. Statistical significance was defined as $P < 0.05$.

RESULTS

Study sample and baseline characteristics

Because of a higher rate of dropouts than anticipated, 318 subjects were finally included in the ITT study sample: 158 in the YCF group and 160 in the CM group (**Figure 2**). This ITT sample consisted of 264 children from Germany (83.0%), 42 from the Netherlands (13.2%), and 12 from the United Kingdom (3.8%). **Tables 2** and **3** show the baseline characteristics and baseline iron and vitamin D status of the 2 treatment groups, respectively. These tables show a higher educational and working status of the parents of the YCF group than the CM group, although more data on this are missing in the CM group than the YCF group. Furthermore, the CM group had a higher iron intake from milk and higher vitamin D intake from food than the YCF group (Table 2).

Figure 2 shows the number of children included in our different study groups and analysis sets. There were no differences in the number of or reasons for early termination (Figure 2) or in the percentage of children demonstrating good compliance (69.6% compared with 71.9%; $P = 0.659$) between YCF and CM users. The aforementioned observed differences in educational status, working status, and iron and vitamin D intake between CM and YCF users were also found in our modified ITT sample and the PP and modified PP sample (data not shown).

Iron status and ID and IDA prevalence

In the (complete) modified ITT sample, the difference in change from baseline in SF between the treatment groups was 6.6 μg/L (95% CI: 1.4, 11.7 μg/L; $P = 0.013$). The estimated mean ± SEM change in SF concentration from baseline was −4.9 ± 2.2 μg/L for the CM group and +1.7 ± 2.4 μg/L for the YCF group (Table 3). We then performed explorative analyses in which the modified ITT sample was divided into 4 subgroups representing categories of most frequently consumed daily volume within the last 4 wk (1–150, 151–300, 301–500, and >500 mL/d). The effect sizes in these subgroups were analyzed while adjusting for sex, country, and baseline SF concentration. In children consuming >500 mL/d, the group difference in change from baseline in SF was 11.2 μg/L (95% CI: 1.8, 20.6 μg/L).

Table 4 shows the prevalence rates of ID and IDA before and after the intervention. The probability of ID after the intervention was lower in the YCF group than the CM group (OR: 0.42; 95% CI: 0.18, 0.95; $P = 0.036$). The IDA prevalence rates were too low to evaluate the effect of the intervention on IDA prevalence.

Hemoglobin concentrations and anemia

At baseline, 18.9% of the children were anemic: 23 in the YCF group and 37 in the CM group. After the intervention, 4 YCF users and 13 CM users were anemic ($P = 0.021$). In contrast, the mean change from baseline in hemoglobin was comparable for YCF and CM users (Table 3).

Vitamin D status and VDD prevalence

In the (complete) ITT sample, the difference in change from baseline in 25(OH)D between the treatment groups was 16.4 nmol/L (95% CI: 9.5, 21.4 nmol/L; $P < 0.001$). The estimated mean ± SEM change in 25(OH)D concentration from baseline was −7.2 ± 2.5 nmol/L for the CM group and 9.2 ± 2.8 nmol/L for the YCF group (Table 3). We then performed explorative analyses in which we determined the effect sizes in subgroups based on the most frequently consumed daily volume within the last 4 wk while adjusting for sex, country, and baseline 25(OH)D concentration. In children who consumed >500 mL/d, the group difference in change from baseline in 25(OH)D was 18.1 nmol/L (95% CI: 3.0, 33.2 nmol/L).

Table 4 shows the prevalence rates of VDD before and after the intervention. The probability of VDD after the intervention was lower in the YCF group than the CM group (OR: 0.22; 95% CI: 0.01, 0.51; $P < 0.001$).

ID and VDD

At baseline, 8.2% of the YCF group and 5.6% of the CM group were iron- and vitamin D–deficient. These prevalence rates increased in the CM group to 15.3% and decreased for YCF users to 4.0% after 20 wk of study product intake.

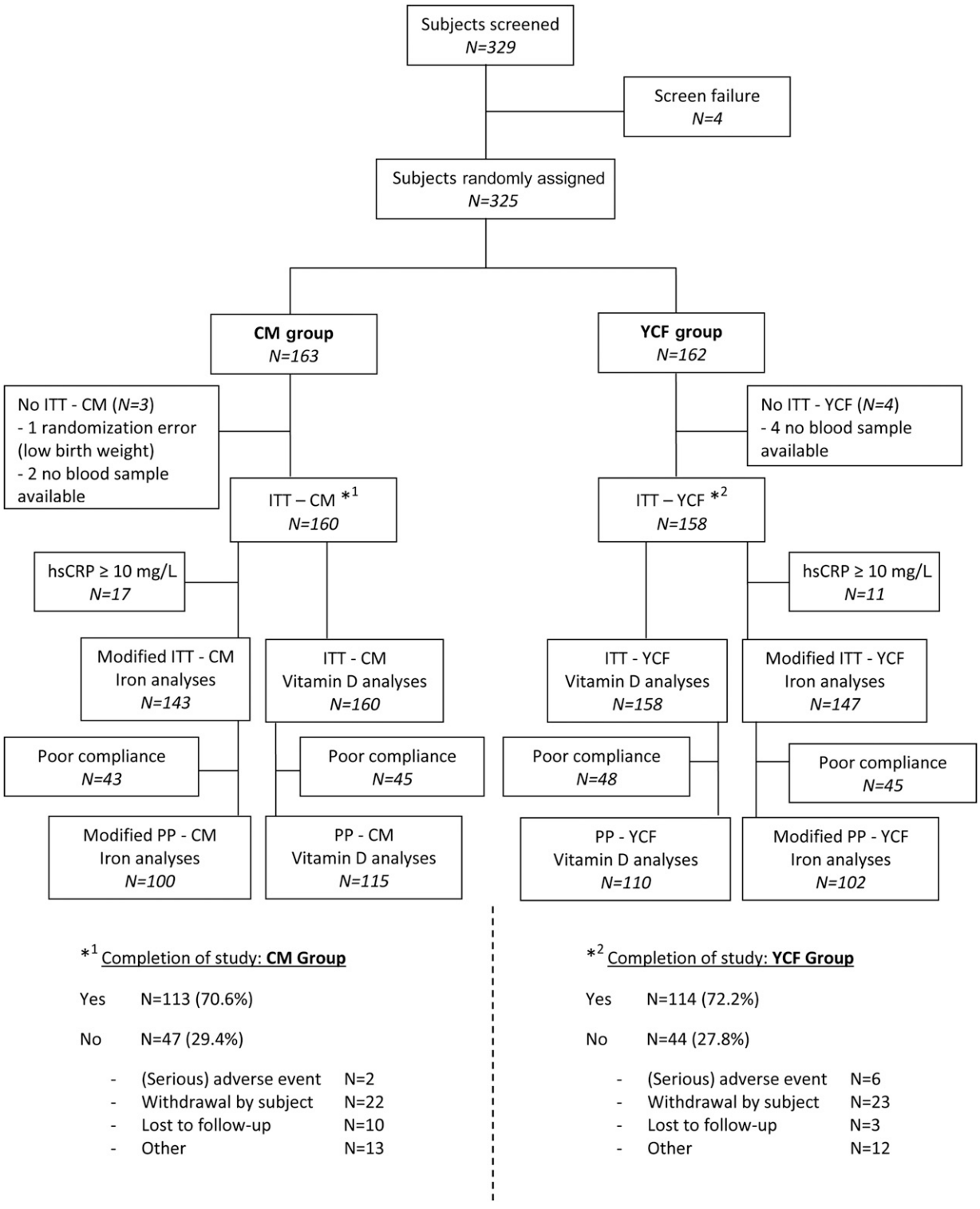

FIGURE 2 Flowchart of the study sample. Children with elevated hsCRP concentrations (≥10 mg/L) were excluded from the analyses regarding iron status to prevent falsely elevated or normal ferritin concentrations in the case of an infection. The PP groups consisted thereafter of children that demonstrated good compliance with instructions for consuming the assigned study product. Good compliance was defined as consuming ≥151 mL study product/d ≥80% of the days within the last 28 d of study product intake. CM, cow milk; hsCRP, high-sensitivity C-reactive protein; ITT, intention to treat; PP, per protocol; YCF, young-child formula.

PP analyses

PP and modified PP analyses confirmed the results from the ITT and modified ITT analyses, although the effect sizes were larger in the PP analyses (data not shown).

Safety of study products: AEs, gastrointestinal tolerance, and growth

Overall, there were no statistically significant differences in the number and severity of reported AEs between the YCF and

TABLE 2
Baseline characteristics of the intention-to-treat study sample[1]

	CM (n = 160)	YCF (n = 158)
Demographic and general characteristics		
Male, n (%)	91 (56.9)	89 (56.3)
Caucasian, n (%)	151 (94.4)	152 (96.2)
Age, mo	20.5 ± 7.72	20.8 ± 7.3
Gestational age, wk	39.0 ± 1.9	39.3 ± 1.4
Birth weight, g	3238 ± 553	3400 ± 513
Educational status of either parent, n (%)		
None	1 (0.6)	0 (0)
Primary school	30 (18.8)	21 (13.3)
High school or trade school	66 (41.2)	80 (50.6)
University	30 (18.8)	35 (22.2)
Unknown	33 (20.6)	22 (13.9)
Professional status of parents, n (%)		
≥1 working	118 (73.8)	126 (79.7)
None working	3 (1.9)	8 (5.1)
Unknown	39 (24.3)	24 (15.2)
Daycare attendance, n (%)		
Yes	75 (46.9)	66 (41.8)
No	84 (52.5)	91 (57.6)
Unknown	1 (0.6)	1 (0.6)
≥1 h spent outside/d, n (%)		
Yes	135 (84.4)	124 (78.5)
No	25 (15.6)	34 (21.5)
Use of sunscreen or protective clothing, n (%)		
Yes	37 (23.1)	51 (32.3)
No	117 (73.1)	103 (65.2)
Unknown	6 (3.8)	4 (2.5)
Characteristics at baseline		
Weight-for-age z score	0.15 ± 0.98	0.28 ± 0.92
Height- or length-for-age z score	0.19 ± 0.99	0.11 ± 1.00
BMI-for-age z score	0.3 ± 1.1	0.3 ± 1.0
Milk intake during previous month		
CM, n (%)	68 (42.5)	73 (46.2)
YCF, n (%)	85 (53.1)	79 (50.0)
Other, n (%)	7 (4.4)	6 (3.8)
Amount per day, mL	517 ± 223	512 ± 230
Use of supplements containing iron, n (%)		
Yes	2 (1.3)	3 (1.9)
No	152 (95.0)	151 (95.6)
Unknown	6 (3.7)	4 (2.5)
Use of supplements containing vitamin D, n (%)		
Yes	51 (31.8)	43 (27.2)
No	103 (64.4)	111 (70.3)
Unknown	6 (3.8)	4 (2.5)
Iron intake at baseline,[2] mg/d		
From milk[3]	3.1 (0.0–5.2)	2.3 (0.0–4.8)
From food	6.8 (5.0–9.9)	6.7 (4.3–10.1)
Vitamin D intake at baseline,[2] μg/d		
From milk[3]	4.4 (0.0–7.0)	4.4 (0.0–6.3)
From food	5.3 (1.1–7.7)	2.0 (0.8–7.0)

[1] Values are n (%) or means ± SDs unless otherwise indicated. CM, cow milk; YCF, young-child formula.
[2] Medians (IQRs) because of no normal distribution.
[3] Includes YCF and CM.

CM groups (data not shown). Of the reported AEs (939 in 258 subjects), 33 events in 27 subjects were considered to be related to the study product. Most of these supposedly related AEs compromised gastrointestinal complaints. There were 30 reports of diarrhea in 26 subjects (17%) from the YCF group and 17 reports of diarrhea in 14 subjects (9.2%) from the CM group (P = 0.061). In both groups, most of these reports were documented in the first week after the start of the study product, and the diarrhea lasted <5 d (data not shown). The complaints were not severe, and most of the complaints were resolved without any medication. Furthermore, there were 9 serious AEs reported in 8 subjects. These events were diverse and evenly distributed over the treatment groups (data not shown). All were considered to be unrelated to the study product.

Table 5 shows the stool characteristics (frequency and consistency) recorded before each hospital or clinic visit (baseline, 10 wk, and 20 wk) by treatment group. No statistically significant differences in gastrointestinal tolerance were observed between the treatment groups. Finally, there were also no statistically significant differences in the anthropometric data between the 2 treatment groups during the intervention period (data not shown).

DISCUSSION

To our knowledge, this is the first randomized, double-blind controlled trial to describe the effect of micronutrient-fortified YCF on both the iron and vitamin D status of healthy children aged 12–36 mo in Western Europe. The results of this study indicate that the daily consumption of YCF for 20 wk preserves iron status and

TABLE 3
Adjusted mean changes in iron and vitamin D status after the intervention[1]

	CM	YCF
Serum ferritin, μg/L		
n	143	147
Baseline	28.9 ± 17.1	25.6 ± 14.8
20 wk	22.0 ± 17.5	27.9 ± 17.4
Change from baseline	−4.9 ± 2.2[2]	1.7 ± 2.4[2,3]
Hemoglobin, g/L		
n	160	158
Baseline	118.5 ± 10.7	119.8 ± 8.8
20 wk	121.9 ± 9.8	123.9 ± 8.2
Change from baseline	3.5 ± 9.1	3.1 ± 8.9
Serum 25(OH)D, nmol/L		
n	160	158
Baseline	70.2 ± 26.7	69.4 ± 27.0
20 wk	62.0 ± 29.9	77.8 ± 26.6
Change from baseline	−7.2 ± 2.5[2]	9.2 ± 2.8[2,3]

[1] Values are means ± SDs unless otherwise indicated. The change from baseline in serum ferritin and serum 25(OH)D were analyzed while adjusting for sex and country (stratification factors), age, micronutrient status at baseline, and the iron or vitamin D intake from food and supplements (and sun exposure in the case of vitamin D). The iron analyses were performed in the modified intention-to-treat sample in which the children with an elevated high-sensitivity C-reactive protein were excluded to prevent falsely elevated or normal ferritin concentrations in the case of an infection. CM, cow milk; YCF, young-child formula; 25(OH), 25-hydroxyvitamin D.
[2] Estimated mean ± SEM (all such values).
[3] The group difference in the change from baseline in serum ferritin and serum 25(OH)D between treatment groups was 6.6 μg/L (95% CI: 1.4, 11.7 μg/L; P = 0.013) and 16.4 nmol/L (95% CI: 9.5, 21.4 nmol/L; P < 0.001), respectively.

TABLE 4
Iron and vitamin D deficiency before and after the intervention[1]

	CM	YCF	OR (95% CI)
Iron deficiency, *n* (%)			0.42 (0.18, 0.95)*
Baseline	17 (11.9)	21 (14.3)	
20 wk	29 (29.6)	14 (13.9)	
Iron deficiency anemia, *n* (%)			—
Baseline	8 (5.6)	4 (2.7)	
20 wk	4 (4.0)	0 (0.0)	
Vitamin D deficiency, *n* (%)			0.22 (0.01, 0.51)*
Baseline	35 (21.9)	40 (25.3)	
20 wk	37 (33.3)	15 (13.5)	

[1] Iron deficiency was defined as serum ferritin <12 μg/L in children without an elevated high-sensitivity C-reactive protein. Iron deficiency anemia was defined as iron deficiency combined with a hemoglobin concentration <110 g/L. Vitamin D deficiency was defined as serum 25-hydroxyvitamin D <50 nmol/L. OR column shows the odds of having iron deficiency and vitamin D deficiency in YCF users compared with CM users. ORs were calculated while adjusting for sex and country (stratification factors), age, micronutrient status at baseline, and the iron or vitamin D intake from food and supplements (and sun exposure in the case of vitamin D). *$P < 0.05$. CM, cow milk; YCF, young-child formula.

improves vitamin D status in young European children. Furthermore, neither study product was related to the incidence of serious AEs. The use of YCF may therefore be an effective and practical strategy for preventing ID and VDD in young European children.

Iron status

We observed a modest increase in SF among the children who consumed YCF. Explorative analyses based on the type of milk before the start of the study (formula or CM) showed a higher increase in SF in original CM users than in original formula users (data not shown). Therefore, young children who consumed CM would probably benefit the most from micronutrient-fortified YCF. One would normally expect a decrease of SF over time because blood volume expands rapidly during growth, requiring increasing erythropoiesis with the use of stored iron and subsequently decreasing SF concentrations (14, 15, 20, 23). Because the SF concentration increased modestly in the YCF group, we suggest that the use of micronutrient-fortified YCF preserves iron stores in young European children.

Four European studies have also reported on the effect of fortified formula on iron status (14, 15, 17, 23), although only 2 (14, 17) used formula with a comparable iron content of 1.2 mg/100 mL. First, Daly et al. (14) investigated the hematologic effects of a follow-on formula in a group of inner-city toddlers whose mothers had already switched to pasteurized CM by 6 mo of age. SF concentrations in formula users remained stable but decreased significantly in the toddlers that continued on CM. The second study that used the same iron dosage focused on the mental and psychomotor developmental indexes at the age of 18 mo after 9 mo of consuming fortified formula. The authors reported significantly higher SF concentrations at 18 mo in the fortified formula users than the nonfortified formula and CM users. Unfortunately, they did not report on SF concentrations at baseline (17). Therefore, the results of our study are only comparable to Daly et al. (14). However, they included younger children, had a longer intervention period, and did not specify details on the ethnicity and socioeconomic status of the participating children. Furthermore, they did not take into account the influence of a possible infection on SF concentrations. These differences in study design make it difficult to compare results.

Vitamin D status

Our observed increase in serum 25(OH)D concentrations in the YCF group is confirmed by a study from Hower et al. (25) among German children. In contrast, Madsen et al. (26) found a decrease in serum 25(OH)D concentration in both formula and CM users in Denmark. In the latter study, a lower fortification dosage of only 0.38 μg vitamin D/100 mL was used compared with 1.7 μg/100 mL in our YCF. This lower fortification dosage may not be sufficient for maintaining adequate serum 25(OH)D concentrations.

The VDD prevalence in our study decreased in the YCF group but increased in the CM group (to 33.3%). In Hower et al. (25), higher prevalence rates ≤79.2% were found in CM users. In this study, the influence of vitamin D–fortified formula (2.85 μg vitamin D/100 mL) on vitamin D status was investigated in children aged 2–6 y. Vitamin D status was determined before and after winter. It is known that the risk for VDD increases during the winter (7, 10), which may explain why VDD prevalence rates were higher than in our study.

Only a minority of the children (∼30%) in our study received vitamin D supplements (mean content: 10.7 μg/d), although policies regarding vitamin D supplementation exist in all 3 participating countries. This emphasizes the need for new strategies, such as the use of fortified food products.

Advantage of fortification with iron and vitamin D (and other micronutrients)

Most of the previously mentioned randomized controlled trials studied the effect of single iron- or single vitamin D–fortified food products. However, a comprehensive review by Best et al.

TABLE 5
Gastrointestinal tolerance: stool frequency and consistency[1]

	CM (*n* = 153)	YCF (*n* = 153)
Stool frequency,[2] stools/d		
Baseline	2 (1–2)[3]	2 (1–2)
10 wk	1 (1–2)	1 (1–2)
20 wk	2 (1–2)	1 (1–2)
Stool consistency[4] (%)		
Baseline	Soft-formed (54.6)	Soft-formed (55.3)
10 wk	Soft-formed (52.8)	Soft-formed (45.0)
20 wk	Soft-formed (57.8)	Soft-formed (47.1)

[1] Analyses were performed in all children from the intention-to-treat sample that actually drank any study product. There were no statistically significant differences between the 2 treatment groups. CM, cow milk; YCF, young-child formula.

[2] Values at baseline were recorded as a single integer; values at 10 and 20 wk were derived from 7 daily frequency values.

[3] Median; IQR in parentheses (all such values).

[4] Presented on an ordered scale as the most frequently recorded stool consistency. The options were watery; soft, pudding-like; soft-formed; dry-formed; and dry hard pellets.

(34) showed that multimicronutrient fortification, such as our YCF, results in more positive effects on biochemical indicators of micronutrient status. In general, it is believed that micronutrients can interact with each other (e.g., by competing for the same transporter) and hereby lead to a different absorption of other micronutrients (34, 35).

For example, ID and VDD seem to influence each other in a negative way, but the precise pathogenesis is unclear (36–39). Vitamin D has been suggested to increase the storage and retention of iron by reducing the activity of proinflammatory cytokines that inhibit iron absorption. On the other hand, it is known that ID impairs the intestinal absorption of fat and the fat-soluble vitamin A and therefore maybe also the absorption of fat-soluble vitamin D. Moreover, iron is a cofactor for the enzyme 1α-hydroxylase, which is responsible for the hydroxylation of 25(OH)D to 1,25$(OH)_2$D (40). Combined fortification of iron and vitamin D may therefore have a synergistic effect on iron and vitamin D status. On the other hand, the bioavailability of iron also depends on the composition of the diet. Food products containing heme iron (e.g., meat) are better absorbed than those containing nonheme iron (e.g., vegetables, milk). Furthermore, several factors enhance (e.g., vitamin C) or inhibit (e.g., calcium) iron absorption. The amount of calcium is lower and the amount of vitamin C is higher in our YCF than in our CM, and this could have also influenced the found effect of our YCF on the change in iron status. Another impact of multimicronutrient fortification is that, in addition to iron, several other micronutrients can also influence hemoglobin concentrations (34).

Safety of micronutrient-fortified YCF

Iron could theoretically increase pro-oxidant stress with potential adverse effects, including infection risk, and possibly affect stool pattern. However, consistent with previous reports (41), we observed no difference either in the frequency and severity of AEs (15) nor in the stool characteristics between YCF and CM users. Consistent with 2 other studies, we also did not find differences in anthropometric variables between YCF and CM users (14, 17).

Strengths and limitations

The strength of our study is that it was a randomized, double-blind controlled trial in a well-defined sample of healthy young Caucasian children in Western Europe. Furthermore, we took into account the influence of infections and the season on iron and vitamin D status variables, respectively.

Most of our study sample consisted of German children, and almost all children were Caucasians. This lack of diversity may hamper generalizing our results to other parts of the world. However, although country and race may influence baseline micronutrient status, we do not believe that it will change the observed effect of our intervention. Another limitation of our study is the use of an adapted food-frequency questionnaire that was not validated for determining iron and vitamin D intake in young children. However, these kind of questionnaires have been found suitable for determining iron and vitamin D intake in infants and preschoolers (42). Finally, the percentage of dropouts (mostly because of nonacceptance of the study product), although similar for both treatment groups, was higher than expected. Approximately 40% of the children consumed CM before the start of the study. During the intervention period, these children were exposed to milk with a different consistency and possibly a different taste. These differences can explain the refusal of some children to drink the study products. Future studies should therefore investigate the best form and taste of fortified YCF.

In conclusion, the daily use of micronutrient-fortified YCF instead of nonfortified CM for 20 wk preserves iron status and improves vitamin D status in children aged 12–36 mo in Western Europe. The current recommendations state that CM is acceptable after the age of 1 y, although the iron and vitamin D intake in these children, including the use of vitamin D supplements, is insufficient for preventing ID and VDD. YCF, as part of a toddler's diet, could play a role in ensuring sufficient intake of certain micronutrients. The long-term benefits of fortified YCF on neurodevelopment and overall health remain to be elucidated.

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