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Vitamin D concentrations in fortified foods and dietary supplements intended for infants: implications for vitamin D intake

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Abbreviated running title: Vitamin D content in fortified foods and dietary supplements

Key words: vitamin D; infant; fortified foods; dietary supplements; analytical measurement

Highlights

- Foods with added vitamin D contained 50-153% of the labelled vitamin D content
- Dietary supplements contained 8-177% of the labelled vitamin D content
- Vitamin D content of 3 of 44 products deviated significantly from EU tolerances
- Trusting label information only may result in invalid estimates of intake

Abbreviations

EFSA, European Food Safety Authority; UL, tolerable upper intake level;

ABSTRACT

Due to potential overages to cover losses during shelf life, the actual vitamin D concentration of fortified foods and dietary supplements may deviate from the label. In this pilot study the vitamin D concentrations of fortified foods (n=29; follow-on formula, baby porridge, curd cheese dessert) and dietary supplements (n=15), both specifically intended for infants, were analytically determined. Compared to the declared values, the vitamin D content ranged from 50-153% for fortified foods and from 8-177% for supplements. In general, both instant follow-on formula and oil-based supplements had a measured vitamin D content similar to or higher than the labelled value. Ready-to-eat baby porridge was the only category in which all measured vitamin D concentrations were below the declared value (74-81%). The use of label information for fortified foods and dietary supplements may result in invalid estimations of vitamin D intake distributions of infants; both under- and overestimation may occur.

1. Introduction

Vitamin D is a fat-soluble vitamin that plays an important role in bone health. In infants, serious vitamin D deficiency leads to rickets (Health Council of the Netherlands, 2012; Institute of Medicine, 2011; The Nordic Council of Ministers, 2014). On the other hand, excessive vitamin D intake is associated with the risk of hypercalcaemia or hypercalciuria and kidney problems. Therefore, several institutes have set tolerable upper intake levels (UL) of vitamin D for several age-groups, including infants (European Food Safety Authority (EFSA), 2012; Institute of Medicine, 2011).

Vitamin D can be synthesized in the human skin upon exposure to ultra violet light. Besides this, food and dietary supplements can be a source. Since it is generally advised to protect young children against direct sunlight exposure to prevent skin cancer, they rely mainly on foods and dietary supplements for their vitamin D. It is difficult to obtain an adequate intake by relying solely on the consumption of vitamin D naturally present in foods (Health Council of the Netherlands, 2012). Similar to other countries

(Health Canada, 2012; The Nordic Council of Ministers, 2014), the Dutch Health Council recommends vitamin D supplementation for young children (Health Council of the Netherlands, 2012). Since 2012 the advice has been to give a daily dose of 10 µg (i.e. 400 IU) supplemental vitamin D to children until the age of 4 years, irrespective of their diet and the actual exposure to sunlight (Health Council of the Netherlands, 2012).

In a previous scenario study it was estimated that 4-11% of the infants aged 7-11 months would exceed the UL (25 µg/day as set by EFSA (European Food Safety Authority (EFSA), 2012)) assuming that all these children would take daily 10 µg supplemental vitamin D as recommended by the Health Council of the Netherlands (Health Council of the Netherlands, 2012). The vitamin D concentration of fortified foods and dietary supplements of that scenario study were mainly obtained using label information (Yetley, 2008). In addition, food composition databases commonly include vitamin D content data for fortified foods and dietary supplements that are derived from labelled values (e.g. Anonymous, 2013; Public Health England, 2015). It is, however, not always possible for food or supplements to contain the exact micronutrient level specified on the label, due to natural and processing variations, as well as changes during storage. But on the other hand substantial deviation from what is labelled could mislead the consumer and should be prevented. The European Commission have provided guidance for setting tolerances for nutrients declared on the label. Tolerances are defined as the acceptable differences between the nutrient values declared on the label and those analysed. For vitamin D, these are -35% to +50% for foods and -20% to +50% for dietary supplements (Anonymous, 2012a). In addition, the added amount should not deviate from the applicable (national) legislation limits (Anonymous, 2006a). In the Netherlands these are, for example, set for follow-on formula (Anonymous, 2006b), baby porridges with grains (Anonymous, 1997), and dietary supplements (Anonymous, 2012b). Little is known about the exact vitamin D content in fortified foods and dietary supplements at the moment of consumption. Some studies indicate that manufacturers of such products may add higher amounts of micronutrients, in general, to compensate for losses during processing and shelf life (so-called overages) (Holick, Shao, Liu, & Chen, 1992; Veatch, Brockman, Spate, Robertson, & Morris, 2005; Yetley, 2007); although, also lower concentrations than labelled are reported (Garg, et al., 2013). The disparity between the labelled amount and the actual composition of a product can be considerable; depending on the nutrient and product measured, concentrations lie in the range of 50-200% of the declared value (Dutch Food Safety Authority, 2009; Yetley, 2007). Consequently, estimations of the habitual intake distribution and evaluations of the prevalence of inadequate intakes or risk of excessive intakes that rely mainly on labelled nutrition content information may be invalid. Therefore, this pilot study aims to investigate the vitamin D concentrations of fortified foods and dietary supplements designed for infants aged 6-12 months and to examine any deviation from the labelled values. In addition, the potential variations of the vitamin D concentrations between different production batches of the same brand and the potential implications for estimation of the vitamin D intake distribution are discussed.

2. Materials and methods

2.1. Product selection

Mid 2014, an inventory was made of vitamin D fortified foods and dietary supplements on the Dutch market especially designed for young children (6-12 months). First, the INNOVA database (www.innovadatabase.com) was searched for foods fortified with vitamin D. In addition, manufacturers' websites were searched and shops were visited to gain insight into the actual supply of vitamin D fortified foods and vitamin D supplements. Vitamin D fortified foods designed for young children were found in the following product groups: infant formula, follow-on formula, baby porridge based on cereals, infant milk, soy milk, yoghurt drink, instant chocolate milk, curd cheese dessert and lemonades. Products not specifically meant for children, such as margarines, were excluded for product analysis and vitamin D supplements in chewable forms were considered as inadequate for infants and therefore the study focused on liquid forms which were found in two types: oil-based and water-based.

In September 2015, 44 products were purchased for this pilot study from local supermarkets and drugstores in the Netherlands: 18 samples of follow-on formula, 10 samples of porridge, 1 curd cheese dessert, and 15 vitamin D supplements (Table 1). This selection included the majority of the available brands and types in each category. Brands in different price categories were included. According to the manufacturer's website, curd cheese dessert is designed for children from one year onwards, but food consumption data showed that curd cheese dessert is also frequently consumed by infants (De Boer, Hulshof, & Ter Doest, 2006). All products were bought within the best-before date. To study a potential difference in vitamin D content between different production batches, pairs of products of the same brand but each with a different best-before date (n=5 follow-on formulas; n=5 dietary supplements) were bought. Thus, 10 different brands of dietary supplements and 13 different brands of follow-on formulas were bought.

2.2. Labelled vitamin D concentration

The labelled vitamin D concentration (in µg) was obtained by the manufacturer's label declaration for comparison with the analysed vitamin D concentration. Label information indicated that 13 out of 29 fortified foods and all 15 dietary supplements contained vitamin D_3. For the remaining 16 fortified foods the information on the label did not specify the vitamin D form. On all labels, one value for vitamin D content was given, with no uncertainty range provided by the manufacturer. Generally, on the labels of follow-on formulas and baby porridges, the vitamin D content was declared per 100 g prepared product. These vitamin D contents were re-calculated to 100 g unprepared product, based on the instructions for preparation on the label. For dietary supplements, the vitamin D concentration was displayed on the label per number of drops per daily dose (i.e. not per mL or g).

2.3. Chemical analysis

The chemical analysis of vitamin D_3 was performed according to European Standards as specified by NEN-EN 12821: *Foodstuffs: Determination of vitamin D by high performance liquid chromatography*

(HPLC) - Measurement of cholecalciferol (D_3) or ergocalciferol (D_2). All analytical work was carried out by TNO Triskelion B.V. (Zeist, the Netherlands), accredited for internal reference method TRIS/VIT/051 to measure vitamin D concentration in all foods. After purchase, the samples were homogenized and aliquoted, thereafter liquid samples were stored at -20 ºC until analysis while powdered samples were stored at room temperature. All samples were analysed in duplicate in unprepared form (without added milk or water) within 3-5 weeks after purchase. To check for saturation during sample reprocessing, the samples of each duplicate had a different weight: 3 and 6 g for solid samples, 10 and 16 g for liquid samples, and 0.25 and 0.5 g for dietary supplements. Saturation during sample processing was not observed in any of the samples.

Vitamin D_2 was used as an internal standard. In short, samples were saponified in 1.5 mol/L alcoholic potassium hydroxide solution for 30 minutes, with added sodium ascorbate and disodium sulfite (antioxidants). Vitamin D_2 internal standard solution was added into the saponification flask. After cooling down, the vitamin D_3 and D_2 were extracted with di-isopropyl ether and purified by solid phase extraction. Vitamin D was fractionated by straight-phase chromatography, subsequently the vitamin D_3 content was detected by ultra performance liquid chromatography (UPLC Waters Quard column acquity HSS C18 SB 2.1*5 mm, 1.8 µm and column acquity HSS C18 SB 2.1*100 mm, 1.8 µm; injection volume 10 µL, flow 0.5 mL/min) using diode-array detection (DAD) at a wavelength of 265 nm. The eluents were A) methanol/acetonitrile/isopropylalcohol (54/44/2) and 4 g ammonium acetate/L, and B) 85% eluent A and 15% milli-Q water and 4 g ammonium acetate/L. The eluents were used in a time gradient 0-5.5 min (30% eluent A; 70% eluent B), 5.6-6.6 min (100% eluent A), and 6.7-9 min (30% eluent A, 70% eluent B). The results confirmed that vitamin D_3 was the only form present in all products. According to the laboratory the combined measurement uncertainty of their method was 13% (= 1 SD); at a level of 3900 IU/kg (i.e. 9.8 µg/100 g). The combined measurement uncertainty included reproducibility, accuracy and homogeneity. The accuracy of the method was high; this indicated that vitamin D was fully extracted (see Table 2 for validation details). The valid range was 2-5000 IU/100 gram or 100 mL. The vitamin D content was expressed in IU (1 µg = 40 IU) per kg unprepared food or supplement.

2.4. Statistical analysis

The vitamin D content was re-calculated to µg per 100 g for solid foods and per 100 mL for liquids using density. As the vitamin D content of dietary supplements on the label is provided per number of drops associated with the daily advised dosage, the weight (g) of 30 drops obtained directly from the dropping bottle provided at purchase was determined (0.751-1.429 g/30 drops depending on brand). Using the weight of 30 drops, the analytically measured vitamin D concentration was re-calculated from IU/kg to µg/advised number of drops. The re-calculated vitamin D concentrations were rounded to two digits.

The measured vitamin D concentrations were compared with the labelled values. The products for which the measured vitamin D value deviated significantly from the declared value were re-analysed in January 2015 (15 weeks after the first analysis). The two-sided 95% confidence interval was calculated as the measured value +/- the combined measurement uncertainty multiplied by $k = 2$ (i.e. coverage factor) (Ellison & Williams, 2012),

The measured vitamin D concentrations were expressed as proportion of the labelled value and compared with the tolerances for nutrient values declared on the label according to EU guidelines (Anonymous, 2012a). According to the rounding guidelines in the EU document, the declared vitamin D content is presented as a value rounded to two significant figures. The first step in the calculation of the tolerance range is the re-calculation of the declared values to a lower and higher value, due to potential rounding of the declared value. For example, for a declared value of 10 µg the lower value is 9.5 µg and the higher value 10.4 µg; for a declared value of 2.0 µg these values are 1.95 and 2.04, respectively. For fortified foods, the lower bound tolerance was calculated as 65% of the lower value (i.e. -35%), the higher bound tolerance was calculated as 150% of the higher value (i.e. +50%). For dietary supplements, the upper bound tolerance was calculated to be identical to that of fortified foods, but the lower bound tolerance was calculated to be 80% of the lower value (i.e. -20%).

Measured vitamin D concentrations were also plotted against the number of days until the best-before date for five paired products of the same brand with different best-before dates, to study the effect of different production batches.

3. Results

3.1. Measured vitamin D concentration and comparison with label information

The summary statistics of the vitamin D concentration split by type of food or supplement are presented in Table 3. The declared vitamin D content varied from 1.0 µg/100 mL for ready-to-eat follow-on formula to 16.5 µg/100 g for instant baby porridge. All supplements had an advised daily dosage of 10 µg. The measured vitamin D content ranged from 4.5 to 21 µg/100 g for instant food products and from 0.9 µg/100 mL to 1.5 µg/100 mL for ready-to-eat foods. The labelled vitamin D content of follow-on formula had little variation compared to instant baby porridge (Fig. 1a and 1b). The measured vitamin D content of dietary supplements ranged from 0.8 to 18 µg per daily advised dosage (Table 3 and Fig. 2).

For the fortified foods, the measured vitamin D content ranged between 50% and 153% of the declared value, whereas for dietary supplements this range was 8% to 177% (Table 3 and Fig. 1-2). In general, both instant follow-on formula (17 out of 18) and oil-based supplements (2 out of 3) had a measured vitamin D content similar to or higher than the declared value. Ready-to-eat baby porridge was the only category in which all measured vitamin D concentrations were below the declared value (74-81%).

3.2. Comparison with EU tolerance limits

The foods with added vitamin D had different declared vitamin D concentrations and as a consequence different tolerance values. Of 2 out of 29 foods the measured vitamin D concentrations were found to be outside the tolerance range. For one follow-on formula (O_03) the measured vitamin D concentration was just above the upper bound tolerance, but not statistically significant (Fig. 1a). One baby porridge (P_05) had a measured vitamin D concentration below the lower bound tolerance, this was borderline statistically significant (Fig. 1b). The tolerance range was also identical for all supplements, as all dietary supplements included in this study had the same declared vitamin D content. Of these dietary supplements, 4 out of 15 had a measured vitamin D concentration outside the tolerance range. For 3 products the measured concentration was lower (S_01, S_02, S_03), for one product higher (S_13; Fig. 2). However, this deviation from the tolerance values was only statistically significant for 2 products, namely S_01 and S_02, which were the same products with a different best-before date.

Re-analyses of the products that statistically significantly deviated from the EU tolerance values resulted in the same conclusion, namely that the values were statistically significant outside this tolerance range (data not shown).

3.3. Difference in vitamin D content of same products from a different production batch

For all but one of the follow-on formulas bought in pairs of the same brand, but from a different production batch (best-before dates 86-333 days apart), the vitamin D concentration was 15-18% higher for the product with the most days until the best-before date (Fig. 3). However, all these deviations were within the measurement uncertainty and not statistically significant.

The dietary supplements did not show a large difference for measured vitamin D concentration between pairs of the same brand but from a different production batch (less than 4%; best-before date 88-365 days apart; data not shown). For one supplement pair (S_01 and S_02) the product with the highest best-before date had a measured vitamin D concentration more than 250% higher than the product with the lower best-before date; however, the vitamin D content for both supplements was very low and deviated significantly from the EU tolerance (Fig. 2).

4. Discussion

To the authors' knowledge , this is the first study to evaluate differences between measured vitamin D concentrations and declared vitamin D contents in fortified foods in the Netherlands. The main finding of the present study is that both lower and higher levels than declared occur in practice; however, in general within the EU tolerance range. Three out of 44 products deviated significantly from the EU tolerance range. Whether the measured vitamin D level is above or below the declared value may be related to the food, for instance instant follow-on formula had a similar or higher measured vitamin D concentration compared to the label, whereas, in the 'baby porridge' group, most measured vitamin D values were similar or below the declared values. For most dietary supplements the measured vitamin D concentration is somewhat above or below the declared value, but larger deviations were also observed in 3 out of 15 products.

For fortified foods, our results are similar to those found in other studies in the USA (Patterson, Phillips, Horst, Byrdwell, Exler, Lemar, et al., 2010; Pehrsson et al.2014; Yetley, 2008), Canada (Nimalaratne, Sun, Wu, Curtis, & Schieber, 2014), and New Zealand (Thomson, 2006). In the USA, the vitamin D content of infant formula ranged from 87-184% of the declared values (Pehrsson et al., 2014; Yetley, 2008), and for whole milk both vitamin D concentrations below and above the labelled value were found, ranging from below the detection limit (i.e. < 0.5 µg/0.95 L (equivalent to a United States liquid quart)) to almost 200% of the declared value (Patterson et al., 2010). In Canada, infant formula was found to contain 70-119% of the labelled values (Nimalaratne et al. , 2014). In New Zealand, 28% of the products (baby food, drinks, margarine and milk products) had vitamin D concentrations below the declared values (47-68%) and the declared values were exceeded in 39% of the products (25-70%) (Thomson, 2006). For dietary supplements the results of the present study are similar to studies in New Zealand (Garg et al., 2013), the USA (LeBlanc, Perrin, Johnson, Ballatore, & Hillier, 2013), Canada (Nimalaratne et al. , 2014) and the Netherlands (Dutch Food Safety Authority, 2009). In the New Zealand study, 9 out of 15 samples had a measured vitamin D concentration within 100% ± 10% of declared value, 3 had a lower measured value (8-29% of declared) and 3 a higher measured value (133-201%) (Garg et al., 2013). In the USA study the potency of the vitamin D content of over-the-counter as well as compounded supplements was highly variable (9-146% of expected) (LeBlanc et al. , 2013), whereas in Canada dietary supplements contained 66-145% of the labelled vitamin D content (Nimalaratne et al. , 2014). In a previous Dutch study the measured vitamin D levels ranged between 50% and 152% of the declared values (Dutch Food Safety Authority, 2009).

In the present study, the combined measurement uncertainty was 13% at a level of 3900 IU/kg (i.e. 9.8 µg/100 g), which is in line with others reporting a relative standard deviation between 15-20% for vitamin D content in supplements (Roseland, Holden, Andrews, Zhao, Schweitzer, Harnly, et al., 2008). It was assumed that the relative combined measurement uncertainty that was provided by the lab for a vitamin D content of 3900 IU/kg is a constant factor over the different dosages measured; however, this may be either an under- or overestimation of the uncertainty (Byrdwell, Devries, Exler, Harnly, Holden, Holick, et al., 2008; Yetley, 2008). The uncertainty in the vitamin D levels of the dietary supplements measured in our study may be even higher because of the re-calculation of the vitamin D concentration per 100 g to a concentration per a certain number of drops, based on the weight of 30 drops, which is not included in the combined measurement uncertainty. The paired samples of the same brand with different best-before dates showed a difference in drop weight in the range of 0-7% (data not shown). The re-measurements of the three products with vitamin D values that differed significantly from the EU tolerance ranges showed large relative differences for two dietary supplements of -29 and 67% (the absolute differences were -0.24 and 2.0 µg/daily advised dose), in which uncertainty in drop weight is included. These supplements were of the same brand and the difference in drop weight was highest for this brand (i.e. 7%). It should be noted that the measured vitamin D value was only 6-50% of the dosage at which the combined measurement error was calculated (i.e. 9.8 µg/100 g). This could be another explanation for the observed higher uncertainty. It was noticed by others that the variability for analytical measurement of vitamin D in dietary supplements was higher compared to most other nutrients which had a relative standard deviation of < 10% (Dwyer, Picciano, Betz, Fisher, Saldanha, Yetley, et al., 2008). Although it could be difficult, a study is recommended to investigate how more consistent analytical results could be obtained for vitamin D. In addition, the EU tolerances for deviations from the declared values are broad for micronutrients added to foods or dietary supplements: +50% or -35 or -20% (food or supplement). In combination with the high combined measurement uncertainties, only very large deviations from the labelled value turn out to be statistically significant deviations from the EU tolerance ranges. Consequently, foods or supplements may contain vitamin D levels much lower or much higher than declared. For pesticides, for example, the tolerances are less broad and range from ± 5% to ±15% depending on how much is declared for homogeneous formulations (FAO/WHO Joint Meeting on Pesticide Specifications (JMPS), 2010). For heterogeneous formulations, the tolerances are expanded to take into account uncertainty in a ratio of the different components in the mixture. Reduction of the measurement uncertainty and/or narrowing the tolerance values may be solutions to decrease the deviation from the declared value. This is not only important for comparison with (legal) tolerance ranges but also for the precision of the concentration data included in dietary assessment and as such the estimation of vitamin D intake (Dwyer et al., 2008; Yetley, 2008).

Besides measurement uncertainty, there might also be other factors that explain the differences between measured and labelled vitamin D values. In general it is supposed that producers apply overages of vitamin concentrations to ensure that the vitamin levels are still available at the end of the shelf life. It seems that this supposition is based on regulations in the USA that state that label values must reflect the minimum amount of vitamin D into the product throughout its entire shelf life (Yetley, 2007). Based on this assumption, a standard overage of 12.5% was applied to all micronutrient contents in fortified foods and dietary supplements in the UK Nutrition Survey Nutrient Databank. For vitamin D this resulted in a 6% increased intake for persons 1.5 years and older (Allen, Dangour, & Tedstone, 2014). Our results indicate that a general overage-correction factor for all types of products seems too simplistic. For example, in our study overages of vitamin D in instant follow-on formula seem on average considerable (126% of declared), whereas in baby porridges the vitamin D concentration was on average closer to the labelled value (92% of declared). This is also supported by others suggesting that under- or over-fortification may cause problems depending on the product type (Byrdwell, Exler, Gebhardt, Harnley, Holden, Horst et al., 2011; Patterson et al., 2010). The prediction formula of actual mean micronutrient contents based on labelled content of the USA Dietary Supplement Ingredient Database (DSID; third release) shows that overages in multivitamin- and mineral supplements differ considerably by micronutrient (Anonymous, 2012c). The basis for these prediction formulas is a regression model of the proportional deviations of actual micronutrient concentrations compared to declared values. For vitamin D levels in multivitamin- and mineral supplements for young children (1-4 years old) the percentage difference from the label for the predicted mean is 38.7% (Anonymous, 2015). In the DSID prediction models, the potential effect of storage was not included. For dietary supplements, our results based on 5 paired samples of the supplements of same brand but from different batches showed no clear relationship between analytically measured vitamin D content and batches with a different best-before date. However, for follow-on formula, four of the five pairs of the same brand, but from different batches, showed a vitamin D concentration deviation of up to 18%. In all of these foods, the product with the longest period until the best-before date showed a higher vitamin D concentration than those products closer to the best-before date. Although this was not statistically significant, this may suggest an effect of storage on the vitamin D concentration. This may indicate that, for these foods, besides declared content, period until best-before date should also be included in a prediction model. A study of the potential variation in vitamin D concentration, between and within different batches in a larger sample, is recommended. In addition, the effect of storage on the vitamin D concentration should be investigated by following the vitamin D concentration during the shelf life-period within the same batch, as well as during normal use (i.e. after opening).

In our pilot study, a limited number of samples were analysed. Variation within batches and during storage was not studied, and the study of variation between batches was limited to a few brands and two batches per brand. It was therefore impossible to develop a valid prediction model for the different food groups. More studies on the potential of a prediction model of micronutrient concentrations in fortified foods and supplements are recommended to improve the quality of the estimation of micronutrient intake. This should also include which additional product information would be required, like the best-before date. Even though this kind of information is currently not known in dietary surveys, this knowledge may help to improve data collection and estimation of dietary intake.

The present finding that the vitamin D contents of fortified foods and dietary supplements may deviate considerably from the declared values may be a concern from a public health perspective. Dietary intake assessment generally relies on label information for fortified foods and dietary supplements (Anonymous, 2013; Public Health England, 2015; Yetley, 2008). These assessments and subsequent evaluations of too low or too high intakes may be invalid and inaccurate. Applying the findings of the present study to a previous study on infant vitamin D intake in the Netherlands provides a preliminary insight in the potential effects. Taking the declared values of vitamin D in fortified foods and assuming daily supplement use of 10 µg/d, the median vitamin D intake of infants 7-19 months of age was estimated to be in the range 13-21 µg/d, and 4-11% of infants aged 7-11 months had an intake above the UL, as set by the EFSA (Verkaik-Kloosterman, Beukers, Jansen-van der Vliet, & Ocke, 2015). Considering the results of the present study in that scenario study, the median vitamin D intake would become 8.4-11 µg/d with none of the infants exceeding the UL if all of the lowest proportional deviations of the label declarations in a studied product category were applied (results not shown). On the other hand, the median intake would become 30-34 µg/d with at least 75% of the infants exceeding the UL if all of the highest proportional deviations were be applied (data not shown). Better estimations of habitual vitamin D intake distributions would require the variability of the actual vitamin D contents in fortified foods and dietary supplements to be taken into account, rather than just the declared values. A combination of a prediction formula with probabilistic approaches (Rubingh, Kruizinga, Hulshof, & Brussaard, 2003) to take into account the precision of the prediction formula parameters is therefore required. We agree with LeBlanc et al. (2013) that, in addition to effects in monitoring studies, the bias and uncertainty resulting from relying on label information for the vitamin D content of fortified foods and dietary supplements, also has an impact on other types of research. In intervention studies, relying on label information only may bias the results due to variability in the nutrient content of fortified foods or supplements. It is recommended that analytical measurements are always performed. Furthermore, in epidemiological studies, the nutrient intake of individuals may be systematically under- or overestimated if only label information is considered and this could have an impact on the association studied. This may also have an effect on dietary reference values, which are set based on such studies. It is important that researchers are aware of the potential deviations of the content compared to the labelled value and the consequences for the interpretation of their results.

5. Conclusions

The vitamin D content of fortified foods and dietary supplements may deviate from the declared value. Although this is in most cases within the tolerance limits for the control of compliance with the declaration on the label, estimations of dietary intake based on label information may be invalid. Depending, for example, on the product, brand loyalty, variability of nutrient content within and between batches and the consumed amounts, both under- and overestimation of the vitamin D intake are possible. Further research is needed concerning the effects of these findings on the habitual vitamin D intake distribution, using advanced statistical approaches like probabilistic modelling and prediction models.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

JVK initiated and designed the study, and was the overall project coordinator. SMS carried out the statistical analyses. JVK, MCO, SMS interpreted the results and wrote the paper. All authors were involved in the interpretation of the results, provided comments and suggestions on the manuscript and approved the final version.

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Figure captions

Fig. 1. Measured vitamin D concentration in A) follow-on formula and B) baby porridge compared to the vitamin D content declared on the label. At the grey line the measured values are equal to declared vitamin D contents, the grey dotted lines show the EU tolerances. Circles with the same letter are identical products with different best-before dates. Only values with a measured vitamin D content outside the EU tolerances are presented with 95% CI. * ready-to-eat products per 100 mL.

Fig. 2. Measured vitamin D concentration (µg/daily advised dosage) in dietary supplements. At the grey line the measured values are equal to declared vitamin D contents (i.e. 10 µg/daily dosage for all), the grey dotted lines show the EU tolerances. Circles with the same letter are identical products with different best-before dates. Only values with a measured vitamin D content outside the EU tolerances are presented with 95% CI; S_01 has 95% CI of 0.64-1.04 µg/daily advised dosage.

Fig. 3. Comparison of measured vitamin D concentrations (µg/100 g) in follow-on formula of the same brand (n = 5) with different best-before dates

Table 1. Overview of the 44 fortified foods and dietary supplements containing vitamin D of which the vitamin D concentration was chemically analysed

Food group	Specification	Number of products	Number of days until best-before date	Labelled age-category
Follow-on formula	Instant milk-based (powdered)	16[a]	68-668	6-10 months or 6-12 months
	Ready-to-eat, milk-based with cereals/yoghurt or fruit (liquid)	2	145-265	> 6 months
Porridge	Instant with cereals (adding milk or water)	7	283-735	> 6 months or > 8 months
	Ready-to-eat, milk-based with cereals	3	148-282	> 6 months
Dessert	Curd cheese with fruit	1	7	> 1 year[b]
Supplement	Water-based, only containing vitamin D	12[a]	85-955	0-4 years
	Oil-based, only containing vitamin D	3	355-895	0-4 years

[a] Including 5 similar products (brand and type) with a different best-before date (interval of 3-12 months)
[b] This product was consumed by infants aged >7 months onwards (De Boer, Hulshof, & Ter Doest, 2006)

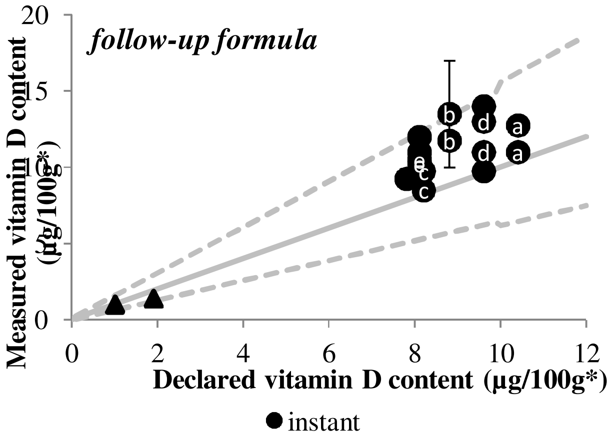
Table 2. Overview of validation parameters of method used to measure vitamin D concentration in infant formula

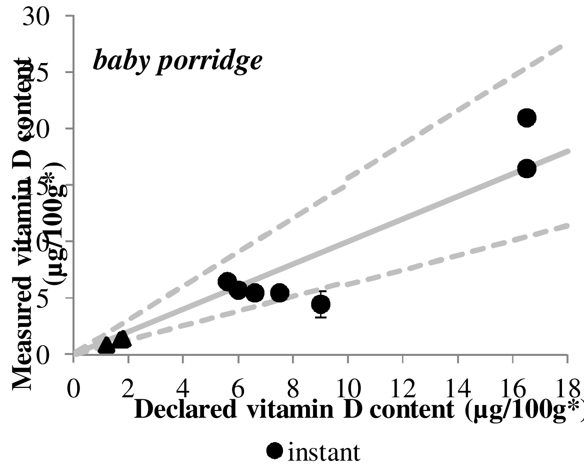
Vitamin D level	Reproducibility (RSD(r))	Repeatability (RDS(r))	Accuracy
20 IU/L			100%
4000 IU/kg	8%	12%	92%
49000 IU/kg	4%	4%	98%

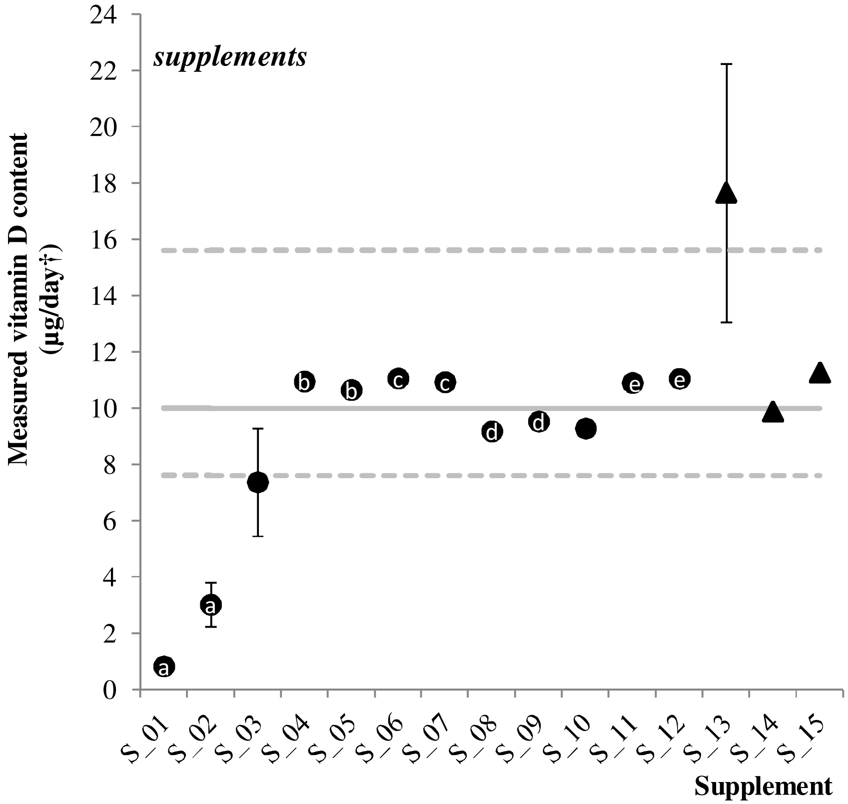
Tabel 3. Overview of labelled and measured vitamin D_3 contents (median; lowest; highest) in fortified foods and dietary supplements and proportion of labelled values (median; lowest; highest)

		number of samples	labelled vitamin D content µg/100 g[a] (number of drops)			measured vitamin D_3 concentration µg/100 g[a]			proportion (%) of labelled value		
			median	range		median	range		median	range	
				low	high		low	high		low	high
Follow-on formula	instant	16	8.5	7.8	10.4	11	8.5	14	125	102	153
	ready-to-eat	2	1.45	1	1.9	1.4	1.4	1.4	87	73	101
Baby porridge	instant	7	7.5	5.6	16.5	5.8	4.5	21	96	50	127
	ready-to-eat	3	1.7	1.2	1.8	1.3	0.9	1.4	78	74	81
Curd dessert		1	1.25	-	-	1.5	-	-	122	-	-
Supplement	water-based	12	10 (5)	10 (4)	10 (10)	10	0.8	11	101	8	111
	oil-based	3	10 (10)	10 (2)	10 (10)	11	9.9	18	113	99	177

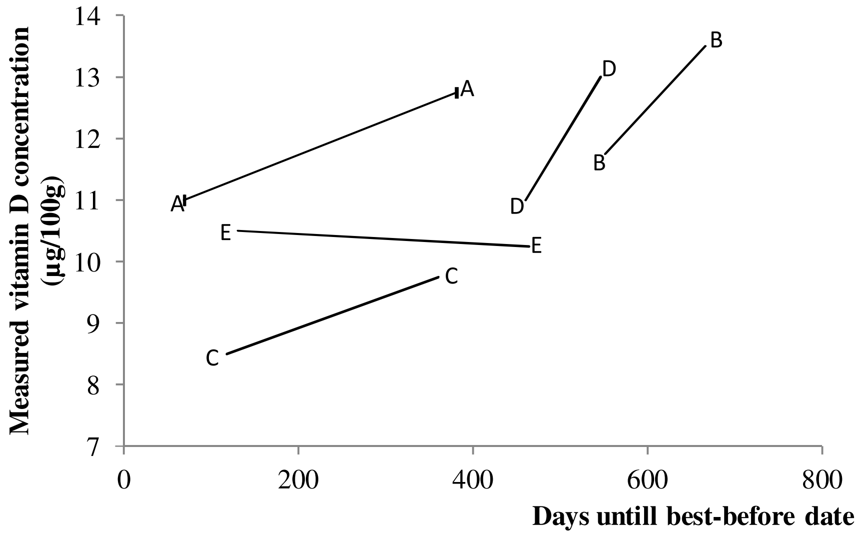
[a] liquid samples per 100 mL, supplements per daily advised dosage provided in amount of drops


follow-up formula
Measured vitamin D content (µg/100g*)
Declared vitamin D content (µg/100g*)
instant
0
2
4
6
8
10
12
0
5
10
15
20
a
b
c
d
e


baby porridge
Measured vitamin D content (µg/100g*)
Declared vitamin D content (µg/100g*)
instant
0
2
4
6
8
10
12
14
16
18
0
5
10
15
20
25
30



supplements
Measured vitamin D content (µg/day†)
0
2
4
6
8
10
12
14
16
18
20
22
24
a
a
b
b
c
c
d
d
e
e
S_01
S_02
S_03
S_04
S_05
S_06
S_07
S_08
S_09
S_10
S_11
S_12
S_13
S_14
S_15
Supplement


Measured vitamin D concentration (µg/100g)
Days untill best-before date
14
13
12
11
10
9
8
7
0
200
400
600
800
A
A
B
B
C
C
D
D
E
E

Highlights

- Foods with added vitamin D contained 50-153% of the labeled vitamin D content
- Dietary supplements contained 8-177% of the labeled vitamin D content
- Vitamin D content of 3 of 44 products deviated significantly from EU tolerances
- Trusting on label information only may result in invalid estimates of the intake