1	Vitamin D ₃ content of cows' milk produced in Northern Ireland and its efficacy as a
2	vehicle for vitamin D fortification: a UK model
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33	Word count for manuscript (excluding abstract, tables, figures and references): 3549
34	Number of figures: 4 Number of tables: 4

35 Abstract

- Cows' milk is a relatively poor source of vitamin D but figures listed in UK food composition tables may be outdated. Samples of milk were collected for 1-year and vitamin D₃
- 38 concentrations analysed using HPLC. Milk consumption data were obtained from the National
- 39 Diet and Nutrition Survey (Years 1-4). A theoretical model applied vitamin D₃ fortifications of
- 40 1µg, 1.5µg and 2µg/100g to simulate improvements in vitamin D intakes. Mean \pm SD vitamin D₃
- 41 in whole milk was $0.06\pm0.02\mu g/100g$. No seasonal differences were apparent. Fortification of
- 42 cows' milks with 1µg, 1.5μ g and 2.0μ g/100g, theoretically increased median vitamin D intakes
- 43 from 2.0µg/day to 4.2µg, 5.1µg and 5.9µg/day, respectively. Higher vitamin D₃ in milk from
- this study than that currently in food composition tables, suggests further analysis is warranted.
- 45 This model suggests vitamin D fortification of cows' milk is an effective strategy to help more
- 46 of the population achieve recently revised RNIs for vitamin D.
- 47

48 Keywords: Vitamin D; fortification; cows milk; food composition; dietary intake; model;
49 NDNS

50 Introduction

Vitamin D plays an essential role in the metabolism of calcium by increasing its absorption 51 in the small intestine (COMA 1991) and for this reason vitamin D has an important role 52 to play in musculoskeletal health (Lanham-New 2008; Pojednic & Ceglia 2014; Todd et al. 2015). 53 Vitamin D deficiency has been known for many years to be a factor in sub-optimal bone 54 health and to lower bone mineral density (Thacher & Clarke 2011). Moreover, poor vitamin 55 D status has been more recently associated with many other non-skeletal chronic conditions 56 such as cardiovascular disease, certain cancers, decline in cognitive function, type II diabetes, 57 and rheumatoid arthritis (Martini & Wood 2009; Kostoglou-Athanassiou et al. 2012; Autier et al. 58 59 2014; Feldman et al. 2014). Vitamin D insufficiency (25-hydroxyvitamin D [25(OH)D] concentration of <50nmol/l) and deficiency (25(OH)D concentrations of <25nmol/l) (IOM 60 2011) are prevalent, with an estimation that 1 billion people could be classed as insufficient 61 or deficient worldwide (Holick 2007). The Irish Adult Nutrition Survey (NANS), reported 62 that approximately one third of adults aged 18-84 y were classed as vitamin D insufficient 63 during the summer, while in the winter this increased to over half of the adult population 64 (IUNA 2011; Cashman et al. 2013). Similar findings regarding vitamin D insufficiency have 65 been noted in the UK National Diet and Nutrition Survey (Public Health England 2014). 66

67 The majority of vitamin D required by humans is derived by ultraviolet (UV)-B radiation 68 of the 7-dehydrocholesterol in the skin (COMA 1991; Webb & Holick 1988), although a number of factors negatively influence the skin's ability to synthesise the vitamin (Hagenau 69 et al. 2009). Such factors include increasing age, skin pigmentation, clothing, 70 sedentary/indoor lifestyles, the use of sun protection and geographical location (i.e. 71 latitude). It has been long established that the northerly latitude of the UK and Ireland [50-72 60°N] means UVB intensity is inadequate to promote the dermal synthesis of vitamin D 73 74 during the winter months (approximately October-March) (Webb et al. 1998; Hill et al. 2008), causing the population to be solely reliant on dietary sources during this time to 75 maintain the body's stores of the vitamin. Despite this reliance on dietary sources, previous 76 77 literature from the UK and Ireland more often than not report low intakes of vitamin D 78 (<5µg/d), because naturally occurring food sources are so limited (IUNA 2011; Public Health England 2014). More worryingly, these figures are considerably lower than the revised 79 reference nutrient intake (RNI) suggested by the Scientific Advisory Committee on Nutrition 80 (SACN) of $10\mu g/d$ of vitamin D daily for the general population aged 4+ y (or the safe intake 81 82 $10\mu g/d$ for children aged 1-4 y) (SACN 2016).

Fortified foods are increasingly contributing to the dietary intake of the population, 83 especially in those who do not also consume dietary supplements (Black et al. 2012). 84 Although milk and dairy products are sources of naturally occurring vitamin D (McCance & 85 Widdowson 2002; BDA 2007), without fortification, the vitamin D content of milk is minimal 86 and has also been known to vary considerably from winter to summer (Kurmann & Indyk 87 1994; Jakobsen & Saxholt 2009). 'The Composition of Foods' series by McCance and 88 Widdowson (2014) provides extensive nutritional data on a number of foods. Although these 89 tables have been updated several times since their inception, the most recent 7th edition 90 (published in 2014), has reported the lowest vitamin D content for whole, semi-skimmed 91 92 and skimmed milk (i.e. as trace) compared to earlier editions, based on limited sampling.

One possible strategy to increase vitamin D intakes within the population is through the 93 94 fortification of milk, which is a staple dietary component for a large proportion of the UK and Irish population. In Canada, the fortification of liquid milk is mandatory at concentrations 95 96 ranging from 0.875µg-1.125µg/100ml (IOM 2011). Indeed, numerous studies have reported the effectiveness of dairy fortification in increasing vitamin D intake in other countries (Calvo 97 98 et al. 2004; Harika et al. 2016; Jaaskelainen et al. 2017). Within the UK, however, mandatory fortification with vitamin D is limited to a few foods only, including margarine, energy-99 restricted foods for diets intended for weight loss and infant formula (Hypponen & Power 100 101 2007; Allen 2015). Vitamin D is only added to a small number of other foods at the discretion of the food industry (e.g. yogurts, cereals and breads). 102

Owing to the low dietary intakes previously reported, and the relatively low uptake of food fortification in the UK/Ireland, alternative food-based strategies to improve consumers' vitamin D intakes, and status are warranted. Therefore, the aims of the current study are to, (1) determine the concentrations of vitamin D_3 in cows' milk produced in Northern Ireland (NI), and; (2) simulate how fortification of cows' milk could theoretically improve overall dietary vitamin D intakes of the UK population using a dietary modelling scenario.

109 Materials and methods

110 Study samples

111 The sampling protocol was designed to be representative of cows' milk on retail sale in 112 NI. Monthly 1L samples of raw and whole pasteurised milk (standardised to a minimum 113 fat content of 3.5%) were collected for a period of 1 year (May 2013 – May 2014) from two 114 dairy processors. All milk samples were collected by staff based within the processing

plants. Raw milk samples were collected immediately pre-pasteurisation. Owing to the well 115 documented seasonal variation in vitamin D content, milk samples collected during October-116 March are referred to as winter milk, while those samples collected between April and 117 September are referred to as summer milk hereafter. Samples were stored at -20°C prior to 118 analysis. Quantification of vitamin D₃ content in stored samples were analysed by HPLC 119 (Agilent 1200 Series) (method adapted from Trenerry et al. (2011). Samples were run with a 120 99% acetonitrile: 1% methanol mix at a rate of 1.5ml per min for 50mins. Vitamin D₃ was 121 quantified at the 265/280 wavelengths. 122

123

124 Population dietary data

The NDNS Rolling Programme Years 1-4 (2008/09-2011/12) dataset was used to provide 125 nationally representative data on both current vitamin D intakes $(\mu g/d)$ and typical milk 126 consumption (g) of the UK population (UK Data Service 2014). The dataset comprises of 3-127 or 4-d food diaries from 4,156 individuals [2,174 adults (18-94 y) and 1,982 children (1.5-128 17 y)]. Consumption of whole, semi-skimmed, skimmed and 1% milks were included in the 129 current analysis. The theoretical impact of vitamin D fortification was evaluated for the 130 entire study population and by age group [children (1.5-17 y old and adults (\geq 18 years)]. 131 132 Attention was also given to sub-groups considered to be at-risk of vitamin D deficiency: young children (aged 1.5-3 y); adults over the age of 65 y (COMA 1991); women of 133 childbearing age (16–49 y) (Public Health England 2014). 134

135 *Dietary modelling*

The vitamin D content of milk as listed in the McCance and Widdowson (2002) was used 136 in the most recent NDNS analysis and therefore acted as the baseline for the current 137 dietary model. Vitamin D fortification concentrations of 1µg/100g, 1.5µg/100g and 138 2µg/100g were selected based on the American and Canadian fortification levels and 139 those of enriched 'super-milks' which are commercially available in the UK and Ireland. 140 These fortification concentrations were then applied to the consumption of whole, semi-141 skimmed, skimmed and 1% milk to estimate the effect fortification at these concentrations 142 143 would have on the overall vitamin D intakes of the population. As part of this dietary model, the current tolerable upper limits (UL) for daily vitamin D intake were considered to 144 determine if the fortification scenario would subsequently give rise to consumer intakes 145 exceeding the UL. The ULs used were those provided by European Food Safety Authority 146

147 (EFSA) at 100µg/d for individuals aged 11+ y, 50µg for children between 1 and 10 y, and
148 25µg for infants <1 y (EFSA 2012).

149 Statistical analysis

The Statistical Package for the Social Sciences (IBM SPSS Statistics 22, Chicago, IL, 150 USA) was used for analysis of all data. Values of P<0.05 were regarded as statistically 151 significant throughout. Normality of the data was assessed using Kolmogorov-Smirnov tests, 152 and where data could not be normalised, the results are expressed as medians (25th and 75th 153 154 percentiles). Descriptive statistics and t-tests were used to describe the study sample and to compare the concentrations of vitamin D present within the different forms of milk between 155 seasons (summer and winter) and between milk types (raw and pasteurised whole) and to 156 compare intakes to the current reference nutrient intake (RNI) (SACN 2016) and tolerable UL 157 (EFSA 2012). The comparison of vitamin D intakes at baseline and post-fortification were 158 tested using non-parametric tests, Wilcoxon Signed Rank test. 159

160 Results

161 Vitamin D analysis of milk

162 The average year-round mean \pm SD vitamin D₃ content of Northern Irish raw and pasteurised whole milk collected as part of this study was $0.08 \pm 0.04 \mu g/100g$ and $0.06 \pm$ 163 164 $0.02\mu g/100g$ respectively, with a range of $0.01-0.16\mu g/100g$ for raw milk (Figure 1) and 0.03- $0.12\mu g/100g$ for pasteurised whole milk (Figure 2). The mean \pm SD vitamin D₃ content per 165 100g between summer vs. winter milk was not significantly different for either raw (0.07 \pm 166 $0.03\mu g$ vs. $0.08 \pm 0.04\mu g$ per 100g; P=0.479) or pasteurised whole milk ($0.07 \pm 0.03\mu g$ vs. 167 168 $0.05 \pm 0.01 \mu g$ per 100g; P=0.227). A significant difference was noted when comparing the vitamin D_3 content of raw and pasteurised whole milk throughout the year (P=0.037). When a 169 seasonal comparison of the vitamin D₃ content of raw and pasteurised whole milk was 170 investigated, a significant difference was noted in winter (P=0.033) but not in summer 171 (*P*=0.506). 172

173 NDNS data

A total of 16,539 recorded dietary days were available for analysis from the raw NDNS dataset [32] of which, 13,962 dietary days (84.4%) reported an intake of milk. Survey

population data can be found in Table 1. Daily milk intakes (portion size per eating 176 occasion) ranged from 2.5g to 2850g. On average, a larger portion size of whole milk was 177 consumed compared to the other three milk types (Figure 3). Semi-skimmed milk was the 178 most commonly consumed milk (Figure 4) in the total study population (53.1% of dietary 179 days). A higher proportion of children (aged 1.5 to 17 y) were consumers of whole milk 180 compared to adults (P < 0.001), and the opposite was true for the other three milk types; 181 however, significant difference was only seen in skimmed milk consumption P<0.001 (semi-182 skimmed P=0.509; 1% fat P=0.505) (Figure 4). 183

Mean dietary vitamin D intake at baseline for the entire study population was 2.50 μ g/day (SD 1.87) with a range of 0.00–20.96 μ g (Table 2). Mean daily vitamin D intakes were significantly higher for males compared to females (2.71 ± 2.09 μ g vs. 2.31 ± 1.65 μ g; *P*<0.001). Adults also had a significantly higher daily vitamin D intake compared to children (2.92 ± 2.13 μ g vs. 2.04 ± 1.42 μ g; *P*<0.001). Baseline vitamin D intakes in at-risk groups are shown in Table 3 and also increased with age.

190 Dietary modelling scenario

191 Of the 4,156 individuals surveyed as part of the NDNS, only 37 (0.89%) met the new 192 RNI of $10\mu g/d$, but following the fortification scenario applied in this dietary model these 193 figures increased. When a fortification of $2\mu g/100g$ was applied 511 (12.29%) of the study 194 population achieved the new RNI (Table 2).

Prior to applying the fortification scenario, six women of childbearing age (0.74%) met 195 196 the RNI of 10µg/d (SACN 2016), following theoretical fortification at 2µg/100g this figure increased to 41 (5.04%) participants. The same increase was seen in those over 65 y, with a 197 198 total number of individuals reporting an intake of 10µg/d or above increasing from seven (1.65%) to 76 (17.76%). The greatest effect of fortification was seen in children (aged 1-3 199 200 y). At the highest fortification, 99 (25.65%) children would be meeting their recommended 201 intake, compared to baseline where only eight (2.12%) were meeting recommended intakes. 202 Up to the highest fortification (2µg/100g), no participants exceeded the age-specific tolerable UL (EFSA 2012), either in the total population (Table 2) or in at-risk groups (Table 203 204 3).

When looking at diary days, fortification was shown to increase the vitamin D intake of the entire population with median intakes increasing from $2.3\mu g/d$ to $6.1\mu g/d$ for semiskimmed milk. For whole milk a similar increase was seen, with a median intake of $1.8\mu g/d$ at baseline and 7.4 μ g/d following fortification at the highest concentration (2 μ g/100g). The effect of simulated fortification at each concentration is shown in Table 4, and fortification at all three concentrations (1 μ g, 1.5 μ g and 2 μ g per 100g) resulted in significantly increased vitamin D intakes for all milk types (*P*<0.001).

212 Discussion

Results from this study clearly demonstrate that a vitamin D fortification policy for milk could potentially help increase the percentage of the population (>12%) achieving the revised RNI/safe intakes of 10 μ g/day vitamin D (SACN 2016). Moreover, even with the highest fortification scenario (2 μ g/100g), no participant within the current fortification model had a vitamin D intake that exceeded EFSA's tolerable UL (100 μ g/d for 11+ y; 50 μ g/d for 1-10 y) (EFSA 2012), suggesting that fortification of milk with vitamin D would be safe in this respect.

A RNI/safe intake of 10µg/d was proposed to ensure that a year-round serum 25(OH)D 220 concentration of \geq 25nmol/l is achieved by the 97.5% of the population (SACN 2016). In the 221 current study, a large proportion of those individuals considered to be 'at-risk' (young children 222 aged 1.5-3 y, women of childbearing age (16-49 y), and those aged 65+ y) fell short of the 223 RNI. Although the fortification model was able to successfully increase the proportion of 224 individuals meeting the RNI, the problem was not completely eliminated. This finding 225 emphasises the importance and need for further strategies to increase vitamin D awareness 226 and intake among these groups, particularly in those who may avoid milk/dairy products as 227 228 part of their habitual diets.

Dietary modelling results similar to those reported by the current study have previously 229 230 been shown by some (Jayaratne et al. 2013; Harika et al. 2016; Ejtahed et al. 2016; Moyersoen et al. 2019) but not others (Allen et al. 2015). In an Iranian population, Ejtahed and colleagues 231 232 (2016) reported an increase in vitamin D intakes from 2.5µg to 3.3µg/d after simulated 233 fortification of milk, which is in line with that reported in the current study for the same 234 fortification (1µg/100g). Jayaratne et al. (2013) also reported a positive effect of a fortification model, with higher increases in intakes shown (3.6 μ g to 6.3 μ g/d), albeit this was achieved by 235 236 fortifying both milk and breakfast cereals so the bigger effect on daily intake is not unexpected. 237

In contrast, negative effects of a milk fortification model on vitamin D intakes were reported in another recent study using UK population dietary survey data. Allen and

colleagues (2015) found that fortification at certain concentrations put a number of 240 participants at risk of exceeding the tolerable UL which is at variance to the current study, 241 even following the highest fortification scenario $(2\mu g/100g)$. This study, however, used older 242 NDNS results collected in fewer participants than used in the current study, and also failed to 243 justify the considerably higher fortification concentrations chosen. Furthermore, the lower 244 values quoted for the tolerable UL of vitamin D intakes were those of the older European 245 Committee report (European Scientific Committee on Food 2002), as opposed to the more 246 recent guidelines from EFSA (2012). 247

The fortification model used in the current study demonstrated an increased vitamin D intake for the entire population, with whole milk having the largest impact on vitamin D intake as a result of the larger portion size consumed per eating occasion. Despite this larger portion size, as semi-skimmed milk was the most frequently consumed milk in the population overall, its fortification would benefit a greater number of people and therefore have the greatest impact on the vitamin D intake at a population level.

The vitamin D₃ concentrations in milk reported in this study are at variance with the results 254 published in some of the latest editions of the McCance and Widdowson (2002; 2014). The 255 7th edition (2014) lists vitamin D for all types of cows' milk as 'trace' with the exception of 256 257 milk from the Channel Islands which is listed at $0.01 \mu g/100g$ (McCance & Widdowson 2014). The previous edition listed the average vitamin D content of whole, semi-skimmed and 258 259 skimmed milk as 0.03µg, 0.01µg and trace per 100g, respectively (Holland et al. 1989; McCance & Widdowson 2002). The increases in vitamin D₃ content of raw and whole milk 260 found in this study, may be as a result of improvements in laboratory methods (Weir et al. 261 2017). Earlier methods of laboratory analysis presented numerous methodological challenges 262 owing to vitamin D's complex structure, often causing complications when extracting the 263 vitamin from the food matrix (Byrdwell et al. 2008) which may also have contributed to the 264 differences in vitamin D₃ content reported. Seasonal variation in vitamin D content in milk 265 across the world has been well documented in the literature (Kurmann & Indyk 1994; 266 Jakobsen & Saxholt 2009) but is not supported by the current study and may be a result of 267 poor weather patterns. In recent years the weather has become more over-cast during the 268 summer months (Sweeney 2016), and this decreases the opportunity for dermal synthesis of 269 vitamin D₃ not only in humans, but also in cattle which synthesise the vitamin in a similar 270 manner (Hymoller & Jensen 2010). Subsequently, the vitamin D status of the cattle influences 271 the vitamin D concentration of the milk produced (Hollis et al. 1981) and therefore, animal 272 husbandry in future should be adapted to ensure a more consistent vitamin D supply 273

throughout the year.

Whilst interpreting the current results, a number of limitations should be noted. First is the 275 use of self-reported dietary intakes, as misreporting in the form of under- or over-reporting of 276 certain foodstuffs is a commonplace in participants (Willet 2013). During the NDNS Rolling 277 Programme, the doubly-labelled water technique was used to validate the reported energy 278 intake (Public Health England 2014) and improves confidence in the data. Moreover, the use 279 of dietary data from the largest nationally representative survey in the UK was the most 280 appropriate to test our hypothesis and such data was considerably more reliable than that 281 282 collected from smaller surveys. Current results are also strengthened by the successful vitamin d fortification programme in Finland (Raulio et al. 2017), and add to the rationale to 283 incorporate fortification in a wide range of food types. Secondly, it was beyond the scope of 284 this project to measure the vitamin D₃ content of all milk types (e.g. semi-skimmed and 285 skimmed), but up-to-date results for the vitamin D₃ content of raw and whole milk from NI 286 have been quantified using a more advanced laboratory technique. Although these values are 287 specific to NI milk, this approach provides novel data on a specific region of the UK, rather 288 than using values from a more widespread and varied pool of data. Owing to the higher 289 290 vitamin D₃ content of milk reported compared to that in the most recent UK Composition of 291 Foods (McCance & Widdowson 2014), a more widespread update of the vitamin D content of UK milk is warranted. It would also be advantageous to use an alternative analytical 292 293 technique, such as liquid chromatography mass spectrometry (LC-MS) (Trenerry et al. 2011) in future studies. This more sensitive method would also enable the quantification of the 294 295 concentrations of other vitamin D metabolites present within milk, e.g. vitamin D₂ and 25(OH)D, which contribute to the total vitamin D content (Cashman 2012). Finally, this study 296 has highlighted the potential beneficial effect of fortifying cows' milk with vitamin D on 297 vitamin D intakes across the UK population. Further analysis should determine how this 298 299 approach would impact the vitamin D contribution from other dairy products (made from the fortified milk), as well as the vitamin D status of the consumer. 300

301 Conclusion

This study suggests that the fortification of UK cows' milk with vitamin D (up to a concentration of $2\mu g/100g$) could be an effective dietary strategy to increase consumer's vitamin D intake, helping more of the UK population to achieve the newly revised RNI for vitamin D of $10\mu g/d$. Importantly, this strategy could translate into a beneficial effect on

- 306 consumer's vitamin D status, without putting anyone at risk of exceeding the tolerable UL for
- 307 the vitamin. Based on the results from this dietary modelling scenario, fortification of all types
- 308 of milk (whole, semi-skimmed, skimmed and 1% milks) is recommended to maximise the
- 309 impact to consumers of all ages and make progress towards eradicating vitamin D deficiency
- among the UK population.

Acknowledgements: The authors would like to acknowledge Alan Beattie for his involvement in the study during his time at AFBI, Dr Maria O'Kane for her assistance with milk collections, and the two milk processors who provided the milk samples in kind for analysis in this study. The authors dedicate this article to the memory of their colleague Professor Julie Wallace (April 7, 1971–February 7, 2012).

Financial Support: The work was supported by a Collaborative Award in Science & Technology (CAST) PhD studentship award funded by the Department of Employment and Learning, and the Dairy Council for Northern Ireland (DCNI). Authors from DCNI contributed to the study design, interpretation of findings and preparation of the manuscript.

320 Authorship: The author responsibilities were as follows: M.J., C.L., A.M.F., J.J.S. and L.K.P

designed the research. R.R.W. and S.S. conducted the research. R.R.W. analysed the data and

322 prepared the manuscript. All authors were involved in interpreting the results, and all read

- 323 and approved the final manuscript.
- 324 **Disclosure statement:** The authors declare no conflicts of interest

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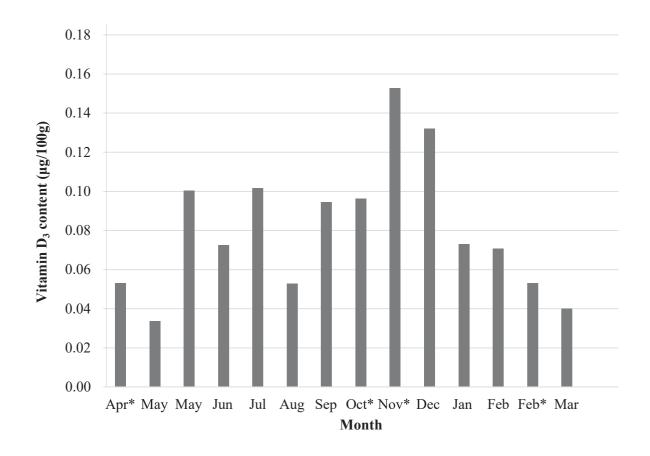


Fig. 1. Vitamin D_3 content ($\mu g/100g$) of raw milk produced in Northern Ireland over a year period. Bars show mean vitamin D_3 of samples collected from two processors across Northern Ireland. *Results available from one processor only.

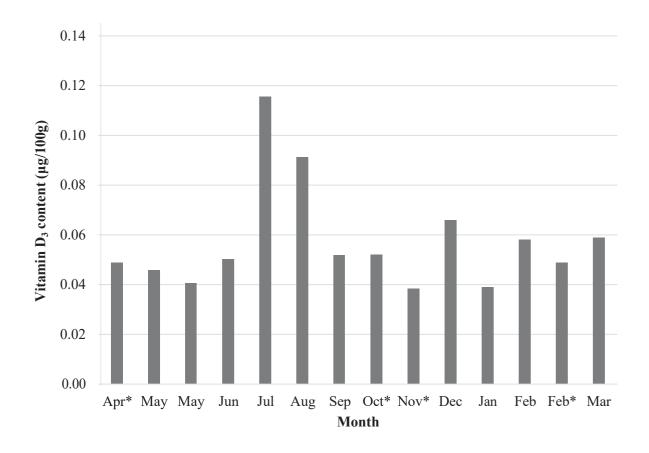
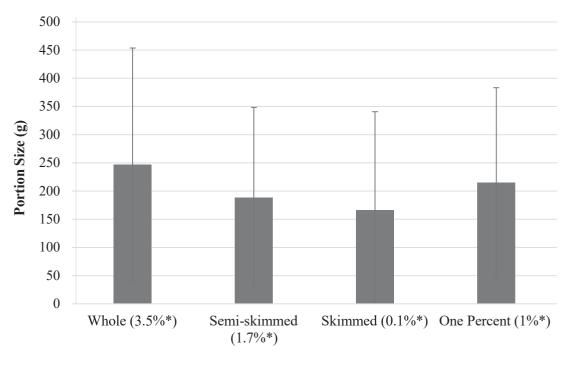


Fig. 2. Vitamin D_3 content (μ g/100g) of pasteurised whole milk produced in Northern Ireland over a year period. Bars show mean vitamin D_3 of samples collected from two processors across Northern Ireland. *Results available from one processor only.



Milk Type

Fig. 3. Mean portion size (g) of milk consumed by participants (n 4,156) per eating occasion for each milk type. *Typical fat content of each milk type.

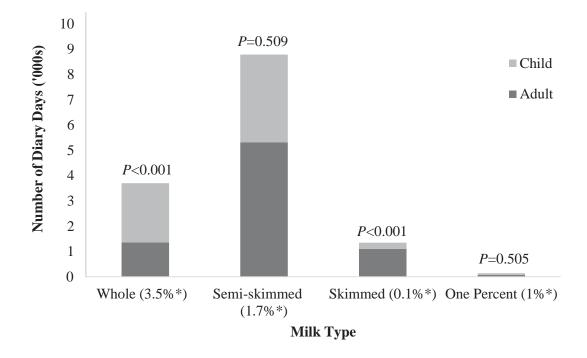


Fig. 4. Frequency of consumption of each milk type by adults (18-94 years; dark bars n 2,174) and children (1.5-17 years; light bars, n 1,982). *Refers to average fat content of each milk type. *P*-values indicate a significant difference between the number of diary days each type of milk was consumed between adults and children (*P*<0.05).

	Sample size (<i>n</i> 4,156)						
Age (yrs)	Male	Female	Total				
1.5 - 3	207	179	386				
4 - 10	414	389	803				
11 - 15	278	265	543				
16 - 18	167	174	341				
19 - 49	471	640	1111				
50 - 64	239	305	544				
≥65	191	237	428				
Total	1967	2189	4156				

Table 1: National Diet and Nutrition Survey (NDNS) population data(Public Health England, 2014)

Table prepared using the demographic information provided in the NDNS report (2014)

	Total Population								
		Vitamin D	Population percentage (%)						
Vitamin D concentration	$Mean \pm SD$	Median	Minimum	Maximum	Meeting RNI ⁺	Exceeding UL [‡]			
No fortification*	2.50 ± 1.87	2.03	0.00	20.96	0.89	(
1µg/100g	4.20 ± 2.48	3.69	0.02	23.94	2.96	(
1.5µg/100g	5.06 ± 3.08	4.42	0.02	33.98	6.88	(
2µg/100g	5.91 ± 3.77	5.11	0.02	44.01	12.29	(

Table 2: Theoretical impact vitamin D fortification of milk on vitamin D intakes of the population based on the NDNS data (*n* 4,156)

NDNS, National Diet and Nutrition Survey, dataset available from the UK Data Archives (2014)

⁺RNI, Reference Nutrient Intake (or safe intake) for vitamin D of 10µg/day for those aged >1 year (SACN 2016)

[‡]UL, upper limit of 50µg/day for those ages 1-10 years and 100µg/day for those over 11 years (EFSA 2002)

*Vitamin D content of milk as listed in the McCance and Widdowson (2002)

Table 3: Theoretical impact of vitamin D fortification of milk on vitamin D intakes of those individuals deemed to be at risk of vitamin

 D deficiency (COMA 1991) based on the NDNS data

	Childr	en aged 1	- 3 years (n	a 386)	Women	of childbe	earing age (<i>n</i> 814)		Adults	s aged over	r 65 years (n 428)	
	Vitamin D) intake	Рорі	lation	Vitamin D) intake	Pop	ulation	Vitamin I) intake	Рор	ulation
	(µg/day)		percentage (%)		(µg/day)		percentage (%)		(µg/day)		percentage (%)	
Vitamin D concentration	Mean± SD	Median	Meeting RNI [†]	Exceeding UL [‡]	Mean± SD	Median	Meeting RNI [†]	Exceeding UL [‡]	Mean± SD	Median	Meeting RNI [†]	Exceeding UL [‡]
	1.96 ±				2.28 ±				3.40 ±			
No fortification*	$\begin{array}{c} 2.05\\ 4.80 \ \pm \end{array}$	1.41	2.12	0	1.65 3.44 ±	1.82	0.74	0	2.39 5.25 ±	2.75	1.65	0
1µg/100g	2.59 6.21 ±	4.42	4.15	0	2.03 4.01 ±	2.99	1.60	0	2.79 6.18 ±	4.69	6.07	0
1.5µg/100g	3.42 7.63 ±	5.53	12.18	0	2.39 4.59 ±	3.50	2.70	0	3.20 7.10 ±	5.50	13.08	0
2µg/100g	4.39	6.87	25.65	0	2.80	3.99	5.04	0	3.70	6.40	17.76	0

NDNS, National Diet and Nutrition Survey, dataset available from UK Data Archives (2014)

⁺RNI, Reference Nutrient Intake (or safe intake) for vitamin D of 10µg/day for those over 1 year (SACN 2016)

[‡]UL, upper limit of 50µg/day for those ages 1-10 years and 100µg/day for those over 11 years (EFSA 2002)

*Vitamin D content of milk as listed in the McCance and Widdowson (2002)

				Total vitamin D		ay)				
	Fortification of milk									
	Not Fortified*		1µg/100g		1.5µg/100g		2µg/100g			
Milk Type	Median	Percentiles	Median	Percentiles	Median	Percentiles	Median	Percentiles		
Whole	2.0 ^a	1.0-3.6	4.9 ^b	3.17.3	6.2 ^c	3.9-9.2	7.4 ^d	4.5-11.2		
Semi-skimmed	2.3 ^a	1.2-4.0	4.3 ^b	2.9-6.6	5.3°	3.5-8.1	6.1 ^d	4.0-9.5		
Skimmed	2.6 ^a	1.2-5.1	4.2 ^b	2.4-7.5	5.2°	2.9-8.5	6.0 ^d	3.1-9.4		
One percent	2.7 ^a	1.4-8.5	4.6 ^b	2.8-9.0	5.5°	3.1-9.1	6.5 ^d	3.8-9.2		

Table 4: Theoretical impact of vitamin D fortification of milk on the dietary vitamin D intake of the population based on reported diary days (*n* 16,539)

Consumption of milk and baseline vitamin D intake as found in the National Diet and Nutrition Survey, dataset available from UK Data Archives (2014)

* Vitamin D content of milk as listed in the McCance and Widdowson (2002) Percentiles (25^{th-}75th)

^{a,b,c,d} Values within a row with different superscript letters are significantly different (*P*<0.001, Friedman Test and Wilcoxon Signed Rank test)