SPi	Journal Code				Article ID					Dispatch: 06.08.16	CE:
	M	C	N		1	2	3	6	8	No. of Pages: 10	ME:

Received: 22 February 2016 | Revised: 2 June 2016 | Accepted: 20 July 2016

DOI 10.1111/mcn.12368

WILEY Maternal & Child Nutrition

ORIGINAL ARTICLE

Calcium to phosphorus ratio, essential elements and vitamin D content of infant foods in the UK: possible implications for bone health

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Abstract

Adequate intake of calcium and phosphorus in the appropriate ratio of 1–2:1 (Ca:P), in addition to magnesium and vitamin D, is vital for bone health and development of an infant. In this feasibility study, the ratio of Ca:P in conjunction with vitamin D and other essential elements (Cu, Fe, K, Mg, Na, and Zn) in a range of commercial infant food products in the UK is investigated. The elemental analysis was carried out using inductively coupled plasma optical emission spectrometry, and vitamin D levels were determined using an enzyme-linked immunosorbent assay. The quantitative data were further evaluated, based on a standardized menu, to measure the total daily intake of an infant aged 7–12 months against the Reference Nutrient Intake. The results from the study show that the Ca:P ratio of the infant's total dietary intake was within the recommended range at 1.49:1. However, the level of intake for each of the nutrients analyzed, with the exception of sodium, was found to be above the Reference Nutrient Intake, which warrants further investigation in relation to both micronutrient interactions and in situations where the intake of fortified infant formula milk is comprised. Finally, as the study is the first to include consumption of infant snack products, the level of total calorie intake was also calculated in order to assess the total daily estimated energy intake; the results indicate that energy intakes exceed recommendations by 42%, which may have implications for obesity.

KEYWORDS

bone health, calcium:phosphorus ratio, commercial infant foods, essential elements, infant snacks, reference nutrient intake, vitamin D

1 | INTRODUCTION

Infancy is a time of rapid growth and development; during which, infants require the correct types and amounts of specific nutrients to ensure optimal growth and development. Typically full term neonates will double their birth weight by 5 months and treble it by the end of the first year of life, in addition to increasing their body length by 25 cm (Gokhale & Kirschner, 2003). During the first year of life, bone mineralization and calcium accretion are greatest (Bass & Chan, 2006). It has been suggested that the calcium to phosphorus ratio (Ca:P) is important for bone growth and development during infancy (Sax, 2001). It is believed that bone mass accumulation in infancy is essential for the prevention of poor childhood growth and adult osteoporosis (Bass & Chan, 2006).

The optimal homeostasis of calcium, phosphorus and magnesium is essential for the formation of the structural matrix of bone; with 99% of calcium and 85% of phosphate present in bone as microcrystalline apatite (National Health and Medical Research Council, 2006). The maintenance of the optimal homeostasis of calcium and phosphorus is also dependent on absorption in the intestine, skeletal accretion and re-absorption and excretion in urine, in addition to vitamin D status and dietary intake (Bozzetti & Tagliabue, 2009).

Extremely low calcium intakes of infants have been associated with rickets even though classically the disease is caused by a nutritional vitamin D deficiency. High phosphorus intakes have been suggested to contribute to hypocalcaemia (low serum calcium levels) and fractures in children (Abrams & Atkinson, 2003); this may in part be due to the actions of parathyroid hormone (PTH) causing re-absorption of calcium and phosphate from the bone; however, further studies are required to evaluate the relationship and mechanisms underlying this proposed effect.

Ca:P may be an important determinant of calcium absorption and retention because of the regulatory mechanisms, which control calcium and phosphorus homeostasis within the body (Bass & Chan, 2006). Animal studies have shown that low Ca:P diets cause low bone densities (Sax, 2001). Common practice is to have a Ca:P molar ratio between 1:1 and 2:1 (Koletzko, Baker, Cleghorn, Neto, Gopalan, & Hernall, 2005). Hypothetically, low Ca:P may adversely affect calcium balance, which subsequently may increase the risk of bone fracture and osteoporosis. Typical western diets are abundant in phosphorus because of the consumption of processed foods; however, calcium intake may be too low (Kemi, Karkkainen, & Lamberg-Allardt, 2006). A high dietary phosphorus intake is suggested to have negative effects on bone through increased PTH secretion, as high serum PTH concentration increases bone resorption and decreases bone formation (Kemi, Karkkainen, Rita, Laaksonen, Outila, & Lamberg-Allardt, 2010).

Other nutrients found in foods can also affect the bio-availability of calcium, for example, zinc and iron (Hallberg, 1998). Therefore, it is important to consider the inter-relation of the nutrients in the diet (Fairweather–Tait & Teucher, 2002).

Formula milk has higher concentrations of calcium and phosphorus but with lower bio-availabilities of both nutrients compared with human milk (Bozzetti & Tagliabue, 2009). In breast milk, the Ca:P is approximately 2:1, with similar ratios in infant formulas; however, absolute quantities are higher in infant formulas to account for the differing bio-availabilities. Breast milk calcium levels remain constant over the first year; however, the phosphorus content decreases over the course of lactation (Bass & Chan, 2006).

In addition, Vitamin D is also important during phases of rapid growth and bone mineralization as in infancy, to ensure optimal calcium balance (Thompkinson & Kharb, 2007). Deficiency of vitamin D in children results in rickets, characterized by skeletal deformity and muscle weakness. Hypovitaminosis D (25(OH)D concentration below or equal to 15 ng/ml) is caused by a combination of inadequate exposure to ultraviolet B radiation and dietary supply. There is a limited supply of natural sources of dietary vitamin D, the highest contributors being fatty fish and eggs. Currently in the UK, fortification of foods with vitamin D is practiced under regulation (EC) no 1925/2006, including breakfast cereals and infant formula products, with mandatory fortification of margarine products (Department of Health, 2011).

Pregnant women, breastfeeding mothers and infants are recommended to use vitamin D supplements; however, according to the 2010 Infant Feeding Survey, only 14% of infants aged 8–10 months were taking vitamin D supplements along with 33% of mothers taking vitamin D supplements at this age. Infant levels of vitamin D usually decline at the weaning period as most foods and cow's milk are low in vitamin D. Between 6 months and 3 years infants and toddlers have an increased need for adequate vitamin D levels because of the high rate at which calcium is being deposited in the bone; they are also susceptible to vitamin D deficiency because of restricted exposure to ultraviolet B radiation from limited outdoor physical activity in day-care centers, low concentrations present in breast milk, and limited intake of vitamin D rich dietary sources (McAndrew, Thompson, Fellows, Large, Speed, & Renfrew, 2012).

The 2008/2009–2011/2012 National Diet and Nutrition Survey survey observed an increased risk of vitamin D deficiency in all age groups of the survey; 7.5% in the 1.5- to 3-year-old group had serum 25(OH)D below 25 nmol/L, a level below, which increases the risk of rickets and osteomalacia. Furthermore, mean intakes of vitamin D from food sources were well below the RNI for the 1.5–3 year old age group (Food Standards Agency, 2014).

This feasibility study investigates the ratio of Ca:P in conjunction with vitamin D and other essential elements (Cu, Fe, K, Mg, Na, and Zn) in a range of commercial infant food products in the UK. In addition, the quantitative data are further evaluated, based on a standardized menu, to measure the total daily intake of an infant aged 7–12 months against the Reference Nutrient Intake (RNI). Finally, as the study is the first to include consumption of infant snack products, the level of total calorie intake is also calculated in order to assess total daily energy intake.

2 | MATERIALS AND METHODS

2.1 | Sample collection for essential elemental analysis

A selected number of dairy-based commercial infant food products representative of four leading brands available on the UK market for infants aged 7–12 months, including eight ready-to-feed infant meals, four infant snacks and one infant breakfast, were obtained from leading supermarkets during June and July 2014. The declared ingredients of all samples and their characteristics are presented in Table 1. Three independent replicates of each sample were analyzed from different food packages, which were purchased from different leading supermarkets in the UK. Samples were stored unopened at room temperature to match the market environment.

Key messages

- The Ca:P ratio of a 7- to 12-month-old infant's diet, based on the consumption of commercial infant foods and infant formula milk, equates to 1.49:1, which is within recommendations.
- The level of intake of essential elements (Ca, Cu, Fe, K, Mg, P, and Zn) exceeds Recommended Nutrient Intake recommendations, which may be due to the inclusion of infant snack products in the diet.
- Total calorie intake exceeds recommendations by 42% for 7- to 12-month-old infants, which may have implications for obesity.

TABLE 1 Ingredients and characteristics of commercial infant food samples for essential elemental analysis from the nutritional label of the food product

Brand code	Product name	Ingredients	Nutritional information (per 100 g)
A	Creamy tomato and leek pasta (7+ months)	Skimmed milk (26%), cooked pasta (durum wheat; 19%), water, carrots (12%), cooked rice (10%), tomatoes (7%), leeks (5%), cheddar cheese (4%), rapeseed oil (1.3%), herbs and spices (rosemary, pepper).	Energy 304 kJ/72 kcal, protein 3.0 g, carbohydrate 8.9 g of which sugars 2.2 g, fat 2.6 g of which saturates 0.9 g, of which linolenic acid (Omega 3) 0.10 g, fiber 0.7 g, sodium 0.03 g.
B	Creamy cauliflower cheese (7+ months)	Baby-grade cauliflower (36%), cooking water, skimmed milk, cheddar cheese (8%), rice, corn starch, parsley.	Energy 263 kJ/63 kcal, protein 3.3 g, carbohydrate 5.8 g of which sugars 1.5 g, fat 2.7 g of which saturates 1.6 g, fiber 1.0 g, sodium 0.07 g.
C	Cheesy tomato pasta stars (7+ months)	Water, tomato (20%), pasta (18%, water, durum wheat semolina), vegetarian cheddar cheese (8%), cornflour, natural flavoring (contains celery, celeriac), iron sulfate.	Energy 285 kJ/68 kcal, protein 2.9 g, carbohydrate 8.5 g of which sugars 0.7 g, fat 2.4 g of which saturates 1.8 g, fiber 0.3 g, sodium 0.1 g, iron 0.9 mg.
D	Cheesy pie (7+ months)	Organic potatoes (25%), organic vegetable stock (24%), (water and organic vegetables: carrots, parsnips, leeks, onions, Swedes), organic sweet potatoes (12%), organic cheddar cheese (10%), organic tomatoes (8%), organic onions (7%), organic carrots (5%), organic broccoli (4%), organic Swedes (4%), organic mixed herbs (<1%), organic peppercorns (<0.01%).	Energy 350 kJ/84 kcal, protein 3.7 g, carbohydrate 8.1 g of which sugars 2.3 g, fat 3.7 g of which saturates 2.2 g, fiber 1.6 g, sodium 0.1 g.
E	Pasta carbonara (10+ months)	Water, cooked pasta (durum wheat; 25%), skimmed milk (21%), cooked rice, onions, ham (5%), grated hard cheese (3%), egg yolk, rapeseed oil (1.5%), herbs and spices (parsley, garlic, pepper).	Energy 372 kJ/89 kcal, protein 4.1 g, carbohydrate 9.3 g of which sugars 1.5 g, fat, 3.8 g of which saturates 1.1 g, of which linolenic acid (Omega 3) 0.13 g, fiber 0.5 g, sodium 0.07 g.
F	Broccoli cheese (10+ months)	Baby-grade vegetables (30%; Carrot, broccoli (8%), onion), potato, skimmed milk, rice (10%), cooking water, cheddar cheese (9%), tapioca starch, black pepper.	Energy 338 kJ/80 kcal, protein 3.9 g, carbohydrate 9.9 g of which sugars 1.7 g, fat 2.8 g of which saturates 1.7 g, fiber 1.3 g, sodium 0.08 g.
G	Cheesy spaghetti with 5 veggies (10+ months)	Water, vegetables (31% carrot, broccoli, onion, parsnip, peas), spaghetti (14% water, durum wheat semolina, egg white), vegetarian cheddar cheese (8%), cornflour, natural flavoring (contains celery, celeriac), iron sulfate.	Energy 333 kJ/79 kcal, protein 3.2 g, carbohydrate 9.8 g of which sugars 3.9 g, fat 2.8 g of which saturates 1.8 g, fiber 1.0 g, sodium 0.1 g, iron 1.0 mg.
H	Spaghetti Bolognese (10+ months)	Organic tomatoes (37%), organic vegetable stock (19%; water and organic vegetables: parsnips, carrots, leeks, onions and Swedes), organic carrots (11%), organic beef (10%), organic broccoli (6%), organic onions (6%), organic spaghetti (5%; durum wheat and egg whites), organic mushrooms (4%), organic cheddar cheese (2%), organic garlic (<1%), organic mixed herbs (<1%), organic peppercorns (<0.01%).	Energy 277 kJ/66 kcal, protein 3.9 g, carbohydrate 6.3 g of which sugars 2.5 g, fat 2.5 g of which saturates 1.1 g, fiber 1.4 g, sodium <0.01 g.
S1	Mini cheese crackers	Organic wheat flour (48%), organic rice flour (19%), organic cheese (14%), organic sunflower oil (8%), organic malt extract (6%), organic malted wheat flour (2%), raising agents (<1%; sodium bicarbonate, ammonium bicarbonate), Thiamin (vitamin B1; <1%).	Energy 1931 kJ/459 kcal, protein 12.1 g, carbohydrate 65.7 g of which sugars 3.9 g, fat 15.8 g of which saturates 7.1 g, fiber 3.1 g, sodium 0.2 g, salt equivalent 0.5 g, thiamine 1.9 mg.
S2	Milk and vanilla cookies (7+ months)	Organic malt extract (27%), organic wheat flour (25%), organic brown rice flour (17%), organic fresh whole milk (12%), organic palm oil (9%), organic wholemeal flour (9%), raising agent (<1%; Sodium bicarbonate), calcium carbonate (<1%), organic vanilla (<0.1%), thiamine (vitamin B1; <0.1%).	Energy 1471 kJ/349 kcal, protein 6.6 g, carbohydrate 55.4 g of which sugars 16.1 g, fat 10.6 g of which saturates 4.9 g, fiber 3.2 g, sodium 0.2 g.
S3	Farley's rusks original	Wheat flour, sugar, vegetable oil, raising agents (ammonium carbonates), calcium carbonate, emulsifier (monoglycerides), niacin, iron, thiamine, riboflavin, vitamin A, vitamin D.	Energy 1737 kJ/411 kcal, protein 7.0 g, carbohydrate 79.5 g of which sugars 29.0 g, fat 7.2 g of which saturates 3.1 g, fiber 2.1 g, sodium 0.01 g, vitamin A 450 ug, vitamin D 10 ug, thiamine 0.53 mg, riboflavin 0.82 mg, niacin 8.8 mg, calcium 390 mg, iron 7.0 mg.
S4	Yogurt (strawberry)	Fromage frais, sugar (8.6%), strawberry puree from concentrate (5%), aronia juice, fructose (1%), modified maize, starch, stabilizers: guar gum, pectin, xanthan gum; flavorings, acidity regulator: lactic acid; vitamin D.	Energy 405 kJ/96 kcal, protein 5.3 g, carbohydrate 12.6 g of which sugars 12.2 g, fat 2.3 g of which saturates 1.6 g, fiber 0.2 g, sodium 0.05 g, calcium 150 mg, vitamin D 1.25 ug.
BF	Multigrain breakfast (7+ months)	Fortified milk (demineralized whey powder, skimmed milk powder, vegetable fat (contains soya lecithin), calcium, vitamins (vitamin C, niacin, pantothenic acid, vitamin E, vitamin B, vitamin B6, vitamin A, folic acid, vitamin K1, vitamin D3, biotin, vitamin B12), iron, zinc, copper potassium, milled cereals (wholegrain wheat, rice, wholegrain millet, wholegrain oats. Skimmed milk powder, dietary fiber (GOS, FOS), demineralized whey powder, rice crispies (rice, corn, whey powder).	Energy 1826 kJ/434 kcal, protein 15 g, carbohydrate 61.9 g of which sugars 37.2 g, fat 12.9 g of which saturates 5.5 g, fiber 5.2 g, sodium 0.1 g, vitamin A 380 ug, vitamin d3 7 ug, vitamin E 2.7 ug, vitamin k1 12 ug, vitamin c 38 mg, thiamine 0.9 mg, niacin 7.5 mg, vitamin b6 0.4 mg, vitamin b12 0.7 ug, folic acid 120 ug, biotin 0.01 mg, pantothenic acid 3 mg, calcium 459 mg, iron 5.6 mg, zinc 2.6 mg, copper 0.2 mg, iodide 104 ug.

2.2 | Sample preparation for analysis of essential elements

A microwave accelerated reaction system (CEM MARS 5®, MARS IP, USA, with XP-1500 vessels), equipped with standard temperature and pressure control systems, was used to digest all samples. Each ready-to-feed baby food sample was mixed and homogenized using a domes- Q4 tic blender (Multi-quick, Braun 300, Havant, UK), and each baby snack was crushed down using a food processor (Vorwerk Thermomix TM31, Bershire, UK). Three independent replicates of 0.5 g (wet weight) were weighed prior to the addition of 5.0 ml of concentrated nitric acid (70% trace analysis grade; Fisher Scientific, Waltham, MA, USA) and 0.5 ml of hydrogen peroxide (30% trace analysis grade; VWR international, Radnor, PA, USA). The samples were then heated for 20 min using microwave digestion, operating conditions shown in T2 Table 2. The digested samples were quantitatively analyzed for eight essential elements (Ca, P, Fe, Zn, Mg, K, Na, and Cu) using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Perkin T3 Elmer Optima 4300 DV, USA), operating conditions shown in Table 3.

2.3 | Preparation of standards for essential elemental analysis

Eight multi-element calibration solutions were prepared at different concentration levels (5–25,000 μg/L) from 1000 μg/L single element ICP grade standards (Inorganic ventures, Christiansburg, VA, USA) using high purity nitric acid (70% trace analysis grade; Fisher Scientific) matched to the sample matrix (10% HNO_3).

A calibration curve, at six concentrations (min 5 ppb – max 25000 ppb), was obtained using these multi-element standards (r^2 = 0.9999).

2.4 | Quality assurance for essential elemental analysis

The accuracy of the analysis was verified by analyzing the Certified Reference Material (NCS ZC73009: wheat), and the concentration for each of the samples was typically within the certified range of T4 ±10% of the certified value shown in Table 4, demonstrating the validity of the method. Blank samples of ultrapure water were also prepared using the same procedures as the samples. Results from the blank controls were subtracted appropriately.

TABLE 3 ICPOES instrument operating parameters applied for determination of essential elements

Parameter	Value
View mode	Variable
View distance	15 mm
Plasma gas flow	15 L/min
Auxiliary gas flow	0.2 L/min
Source equilibration time	15 s
Pump flow rate	1.50 ml/min
Detector	Segmented array change coupled device
Power	1300 watts
Nebulizer	0.80 min
Sample aspiration rate	1.50 ml/min
Read	Peak area
Number of replicates	3
Background correction	2-point
Read delay	60 s
Rinse delay	30 s

TABLE 4 Measured results (*mean (n = 5) and RSD), **certified values (mean ± uncertainty) and % recovery for Certified Reference Material (NCS ZC73009: wheat) to determine quality assurance of ICPOES method for essential elemental analysis

Element	*Measured (mg/kg)	**Certified (mg/kg)	% Recovery
Ca	319.62 (3.07)	340.00 ± 20	94.01
P	1331.38 (0.98)	1540.00 ± 70	86.45
Fe	14.52 (1.32)	18.50 ± 3.1	78.49
Zn	10.92 (2.28)	11.60 ± 0.7	94.14
Mg	377.60 (1.59)	450.00 ± 70	83.91
K	1280.00 (2.35)	1400.00 ± 60	91.43
Na	10.80 (12.45)	17.00 ± 5	63.53
Cu	2.52 (1.77)	2.70 ± 0.2	93.33

*Average of measured Certified Reference Material values (n = 5) and relative standard deviations (RSD). **Certified Reference Material values (NCS ZC73009: wheat) ± uncertainty.

2.5 | Sample collection for vitamin D3 analysis

Because of limited availability of food sources rich in vitamin D, a different range of food samples was selected for the vitamin D analysis, on the basis of their ingredients that are known to be rich in vitamin D,

Q5 **TABLE 2** CEM MARS 5®, (XP-1500 vessels) microwave digestion conditions* for essential elemental analysis

Microwave conditions	Nitric acid digestion of semi-solid samples
Sample	0.5 g
Nitric acid (HNO_3)	5 ml
Hydrogen peroxide	0.5 ml
Pressure†	Max 400 psi
Power	1200 W – 100%
Temperature‡	Step 1: ramp to 190°C over 20 min. Step 2: Hold at 190°C for a further 5 min; allow to cool at room temperature for 1 h.

*Microwave conditions and digestion procedures were adapted and modified based on the CEM operation manual (674007 version). †The electronic pressure sensor (EST & ESP-1500) were used to control and monitor the conditions inside the vessels to avoid exothermic reaction and over-pressurization of the digestion vessels. ‡The electronic temperature sensor (EST & ESP-1500) were used to control and monitor the conditions inside the vessels to avoid exothermic reaction and over-pressurization of the digestion vessels.

such as cheese, fish, and eggs. Four infant meal products were purchased from leading supermarkets in the UK between June and July 2014. The list of the ingredients of the baby food samples and their characteristics are presented in Table 5. The sample jars were stored unopened at room temperature, similar to their distribution and market environment. Three independent replicates of each sample were analyzed from different food packages, which were purchased from different supermarkets in the UK.

2.6 | Sample preparation for analysis of vitamin D3

The current analytical methods for vitamin D analysis are time consuming, labor intensive, require experienced analysts, and have only been validated for a few materials. The official methods available are relatively similar and involve saponification and extraction, clean-up steps and separation using high-performance liquid chromatography, and detection with diode array, with relative standard deviations between 10% and 15% (Byrdwell, DeVries, Exler, Harnly, Holden, & Holick, 2008). In this study, analysis of vitamin D3 was performed using Vitakit D™ (SciMed Technologies, Canada, USA), which is a competitive enzyme immunoassay kit. The enzyme-linked immunosorbent assay (ELISA) could detect vitamin D3 between 0.125–0.75 IU/ml, where no sample in our analysis fell outside this detectable range, and the intra assay relative standard deviations for the ELISA was 6.8%.

Each of the food samples were diluted with deionized water to a fat content of 1–3%, and then mixed and homogenized using a domestic blender (Multi-quick, Braun 300, Havant, UK), and three independent replicates of 1 g (wet weight) were weighed prior to the addition of 0.55 g of potassium hydroxide (laboratory reagent grade; Fisher Scientific) into 15 ml centrifuge tubes. The tubes were gently mixed and left uncapped for 2 min in the dark. The tubes were then capped and incubated in the dark for 4 min, followed by 2 min of vigorous shaking; this step was repeated twice. 2 ml of hexane (high-performance liquid chromatography grade; Fisher Scientific) was then added to the tubes, which were then capped and shaken vigorously for another 2 min in the dark. Centrifugation at 3500 RCF for 10 min at room temperature was then performed. 200 μl of the upper organic phase was transferred to an amber screw cap glass vial.

10 μl of calibrators, extracted samples and controls were pipetted into the ELISA plate accordingly. The plate was shaken for 8 min on a plate shaker (180 ± 10 rpm) to evaporate the hexane. 60 μl of assay buffer was added to each well and mixed gently for 30 s. A lid was placed over the plate and shaken for 5 min (180 ± 10 rpm). 60 μl of anti-vitamin D3 conjugate with horseradish peroxidase diluted in conjugate diluent was added to each well and gently mixed for 20 s. The plate was covered and shaken for 10 min in the dark (180 ± 10 rpm). A microplate washer (Labtech, LT-3000, East Sussex, UK) was used to wash the plate six times with 380 μl/well of distilled water. After washing, the plate was tapped against absorbant paper until no trace of water was visible on the paper. 60 μl of substrate was added to each well and gently mixed for 10 s. The plate was then incubated in the dark for 5 min. Finally, 60 μl of stopping solution (0.2 M H_2SO_4) was added to the plate and gently mixed for 10 s. A Microplate Reader (Thermo Fisher Scientific, Multiskan Ascent, MA, USA) was used to measure the absorbance at 450 nm immediately.

2.7 | Preparation of standards for vitamin D3 analysis

Five concentrations of vitamin D were supplied with the kit; ranging from 0 to 0.75 IU/ml. A calibration curve was obtained with a correlation coefficient of 0.9814.

2.8 | Quality assurance for vitamin D3 analysis

Two control concentrations were supplied with the VitaKit D, 0.2 and 0.6 IU/ml. Analytically obtained concentrations were typically ±10%, demonstrating validity of the method.

2.9 | Estimation of total daily intake

A standardized menu approach has been implemented to estimate the total daily intake of an infant aged 7–12 months, which has previously been proposed by Zand, Chowdhry, Pullen, Snowden, and Tettah (2012a), to take into consideration the consumption of commercial infant foods tested in this study and commercial infant formula. Using the gastric capacity of an infant (30 g/kg body weight/day) with the average weight of an 8-month-old infant (8.3 kg), an infant requires

TABLE 5 Ingredient and characteristics of commercial infant foods for vitamin D3 analysis from the nutritional label of the food product

Brand code	Product name	Ingredients	Nutritional information (per 100 g)
VD1	Cheesy fish pie (7 M+)	Cheese sauce (33%, skimmed milk, cornflour, vegetarian cheddar cheese [2%, contains milk]), water, vegetables (27%, cauliflower [10%], broccoli, potato, onion), hake (8%, fish), iron sulfate.	Energy 192 kJ/45 kcal, Fat 0.7 g, of which saturates 0.4 g, Carbohydrate 6.2 g, of which sugars 1.9 g, fiber 0.6 g, Protein 3.2 g, salt 0.08 g, Sodium 0.04 g, Iron 1.1 mg
VD2	Creamy fish pie meal (7 M+)	Vegetables (52%, peas [12%], potato [10%], carrot [10%], sweetcorn, onion), water, Alaska Pollock (8% fish), cheddar cheese (6%, milk), skimmed milk powder, cornflour, parsley, iron sulfate.	Energy 359 kJ/85 kcal, Fat 2.2 g, of which saturates 1.3 g, Carbohydrate 10.0 g, of which sugars 3.6 g, fiber 2 g, Protein 5.4 g, salt 0.21 g, Sodium 0.1 g, Iron 1.0 mg, Calcium 80 mg
VD3	Pasta bake with tuna (7 M+)	Cheese sauce (water, whole milk, cornflour, vegetarian cheddar cheese [contains milk]), vegetables (21%, tomato [11%], sweetcorn, carrot), pasta (14%, water, durum wheat semolina), tuna (8%, fish), iron sulfate.	Energy 277 kJ/66 kcal, Fat 1.3 g, of which saturates 0.8 g, Carbohydrate 9.6 g, of which sugars 1.2 g, fiber 1.0 g, Protein 3.4 g, salt 0.11 g, Sodium 0.05 g, Iron 1.6 mg
VD4	Egg Custard (4–6 M)	Skimmed milk (30%), full cream milk (30%), rice (29%), sugar, water, egg (3%), nutmeg (0.1%).	Energy 332 kJ/79 kcal, Fat 1.3 g, of which saturates 0.7 g, Carbohydrate 13.5 g, of which sugars 8 g, fiber 0.8 g, Protein 2.8 g, salt 0.1 g

249 g/day from foods (Scientific Advisory Committee on Nutrition, 2011). However, for the elemental analysis, the gastric capacity has been divided by four to allow 25% for breakfast (62.25 g), 50% for lunch and dinner (124.5 g), and a further 25% for snacks (62.25 g) based on the infant food products described in the sample collection for essential elemental analysis section. The estimated amount for milk consumption has been set to 600 ml as recommended by the manufacturer's labeling of infant formula. The concentrations of elements and vitamin D from infant formula have not been analytically quantified in this study; the values have been calculated based on average concentrations provided by the manufacturer's label from leading brands of infant formula available in the UK. The total daily intake is finally calculated by adding the contribution from infant formula and from the foods analyzed in this study; this value can then be compared with the RNI to ascertain whether infants are meeting recommendations based on the proposed standardized menu.

2.10 | Estimated energy intake

The daily estimated energy intake was calculated based on the nutritional labeling information provided by the manufacturer, for infant food products from the sample collection for essential elemental analysis section and commercial infant formula. Taking into consideration the energy contribution from the commercial infant formula (600 ml), 62.25 g for breakfast and snack products and 124.5 g for infant meal products to ascertain whether infants are meeting energy requirements (estimated average requirement [EAR]) based on the proposed standardized menu.

2.11 | Statistical analysis

The experimental results were subject to statistical analysis using Excel 2010 and SPSS package v.17.0. Means and coefficient of variation of the data are presented. The data were further subjected to analysis of variance (ANOVA) at p = .05 to examine the differences between replicated (n = 3) measurements.

3 | RESULTS AND DISCUSSION

3.1 | Essential elements

This feasibility study investigates the Ca:P ratio of an infant's diet based on the consumption of commercial complementary infant foods. The concentration of eight essential elements, in eight infant food products, four infant snacks, and one infant breakfast product, targeted for infants aged 7–12 months were determined by using ICP-OES. The results obtained are presented as per 100 g of the food samples in Table 6. The results of the essential elemental were further subjected to two factor ANOVA without replication analysis. The calculated F value for the ANOVA within groups (between the replicates) showed no significant difference with p values calculated (calcium p = .11, phosphorus p = .06, iron p = .36, zinc p = .83, magnesium p = .11, potassium p = .19, sodium p = .32, and copper p = .07 for meals; calcium p = .60, phosphorus p = .93, iron p = .46, zinc p = .18,

TABLE 6 Concentration of essential elements (Ca, P, Fe, Zn, Mg, K, Na, and Cu) in commercial infant foods determined by ICPOES (mean [n = 3] and CV, coefficient of variation)

	Brands	Ca (mg/100 g)		P (mg/100 g)		Fe (mg/100 g)		Zn (mg/100 g)		Mg (mg/100 g)		K (mg/100 g)		Na (mg/100 g)		Cu (mg/100 g)	
		n (3)	CV	*n* (3)	CV	*n* (3)	CV	*n* (3)	CV	*n* (3)	CV	*n* (3)	CV	*n* (3)	CV	*n* (3)	CV
Meals	A	77.88	.04	58.09	.03	0.28	.10	0.35	.03	11.58	.01	128.78	.02	32.67	.01	0.04	.61
	B	109.01	.00	78.16	.02	0.12	.13	0.50	.03	9.05	.08	86.94	.02	72.93	.02	0.02	.00
	C	68.14	.02	55.27	.01	1.26	.03	0.42	.01	8.88	.01	78.01	.02	97.90	.02	0.03	.00
	D	88.09	.03	67.23	.02	1.20	.18	0.42	.00	13.22	.03	186.71	.03	72.53	.03	0.07	.11
	E	62.09	.03	64.91	.03	0.31	.00	0.42	.04	9.21	.02	76.39	.01	66.41	.00	0.05	.20
	F	87.50	.04	74.38	.06	0.16	.00	0.47	.03	10.91	.05	161.61	.04	76.35	.04	0.02	.50
	G	68.79	.03	54.90	.02	1.17	.10	0.37	.08	9.36	.01	80.72	.01	83.43	.02	0.02	.25
	H	35.46	.04	51.98	.02	0.79	.03	0.59	.03	13.10	.04	237.04	.04	37.48	.03	0.12	.06
	Mean	74.62		63.12		0.66		0.44		10.66		129.52		67.46		0.04	
Snacks	S1	217.30	.01	247.62	.02	1.56	.03	1.74	.02	38.17	.02	161.03	.02	175.49	.02	0.42	.01
	S2	187.28	.03	196.35	.01	1.19	.06	0.97	.02	56.84	.01	287.37	.00	171.35	.01	0.16	.04
	S3	564.02	.01	77.72	.02	9.17	.06	0.76	.01	22.37	.02	147.50	.02	2.57	.19	0.11	.00
	S4	160.82	.04	109.63	.03	0.04	.20	0.61	.03	11.80	.03	134.46	.03	30.30	.05	0.02	.00
	Mean	282.35		157.83		2.99		1.02		32.29		182.59		94.93±		0.18	
	BF	437.25	.02	322.26	.02	5.17	.02	2.15	.03	56.05	.02	532.63	.02	120.21	.03	0.19	.00

magnesium p = .52, potassium p = .06, sodium p = .27, and copper p = .42 for snacks), which indicates the consistency of measurements.

Although the data are insightful, it is important to examine the entire daily nutrient intake when studying the nutrient quality of complementary infant foods in order to ascertain the suitability of these products in relation to dietary recommendations. Therefore, the results shown in Table 6 were further analyzed to estimate the total daily intake of a 7- to 12-month-old infant based on a standard feeding regime suggested by Zand et al. (2012a), which is demonstrated in Table 7. The total daily intake in Table 7 is based on the formula milk contribution of an infant (600 ml as recommended by COMA for a 6- to 9-month-old infant) as well as the gastric capacity of an average 8-month-old infant (30 g/kg of body weight) in order to ascertain the nutritional value of these products in relation to the RNI. The gastric capacity of an 8-month-old infant, with an average weight of approximately 8.3 kg is estimated to be 249 g/day, which ideally should be divided by three to make up breakfast, lunch, and dinner (Zand, Chowdhry, Wray, Pullen, & Snowden, 2012b). In this particular study, the gastric capacity has been divided by four to allow 25% for breakfast (62.25 g), 50% for lunch and dinner (124.5 g), and a further 25% for snacks (62.25 g).

The calculated Ca:P ratio was 1.49:1 (Table 7), which is within the recommended range of 1:1–2:1 (weight/weight) by the European Society for Paediatric Gastroenterology Hepatology and Nutrition to ensure optimal bone health and development (Koletzko et al., 2005). However, the estimated total daily intake for calcium and phosphorus was 924 and 618 mg/day, respectively (Table 7), which equates to 176% and 155% above the RNI, respectively. It is important to note that the aforementioned is in agreement with previous studies carried out by Skinner, Carruth, Houck, Coletta, Cotter, & Ott (1997); Butte, Fox, Briefel, Siega-Riz, Dwyer, & Deming (2010); and Melo, Gellein, Evje, and Syversen (2008). In these studies, the estimated daily intakes of calcium and phosphorus are also shown to be above the RNI for infants. The study herein however demonstrates the highest value for the calcium and phosphorous intake, which could be due to inclusion of infant snack products being investigated for the first time.

Although the concentration of calcium is below the National Institute of Health (NIH) tolerable upper intake level (UL) of 1500 mg/day and, therefore, does not pose any risk of exposure in relation to renal insufficiency and vascular and soft tissue calcification (Institute of Medicine, 2011); it still warrants further investigation because of the inhibitory impact on iron and zinc bioavailability.

All of the snacks and breakfast infant food products were higher in concentration than the ready-to-feed meals in all micronutrients probably because of fortification (with the exception of sodium); furthermore, when the total daily intake does not include the breakfast or snack products, all essential elements typically are within ±10% of the RNI. Therefore, it is important for parents to select breakfast and snack options that are nutritionally adequate for the infant's diet and to not exceed recommendations when added to the infant's diet. More attention needs to be focused upon infant snacks as national surveys have shown that snacking increases with age and that a higher percentage of 12- to 18-month-olds snack on 'sugar preserves and confectionary' (63%) compared with 'savory snacks' (43%), and there is currently limited data available in relation to their nutritional suitability (Hardwick & Sidnell, 2014).

Infant formula alone contributes 73.4% of Ca, 60.8% of P, 80.8% of Fe, 80.2% of Zn, 43.7% of Mg, 66.6% of K, 39.0% of Na, and 76.7% of Cu of the RNI, which for most elements is a high percentage, mainly because of formula being fortified. Therefore, if infant formula milk intake is compromised or breast milk concentrations are low because of poor maternal nutrition, the infant may be at risk of deficiency.

The other important factor to bear in mind is the issue of nutrient interaction and the impact on bio-availability. The consumption of elements therefore cannot be considered in isolation because of their interferences with digestion and absorption (Sandström, 2001).

The issue of bioavailability has a high relevance when considering that the intake of all the micronutrients tested herein, based on the standardized menu, is in excess of the RNI (Table 7), with the exception of sodium, because of sodium being replaced by potassium in many foods following the Food Standards Agency legislation on reduction

TABLE 7 Total daily intake of essential elements (Ca, P, Fe, Zn, Mg, K, Na, and Cu) by an infant age 7- to 12-month old*, based on the gastric capacity of an 8-month-old infant and the standard feeding regime composed of commercial infant food products and infant formula milk in relation to the reference nutrient intakes (RNI)

	Infant formula		Breakfast		Meals (lunch and dinner)		Snacks		Total daily intake	RNI (7–12 months)	% RNI
Element (mg)	100 ml	600 ml†	100 g	62.25 g‡	100 g	124.5 g§	100 g	62.25 g¶	mg/day	mg/day	
Ca	64.25	385.50	437.25	272.19	74.62	92.90	282.35	175.77	926.36	~~525~~	176.45
P	40.50	243.00	322.26	200.61	63.12	78.58	157.83	98.25	620.44	~~400~~	155.11
Fe	1.05	6.30	5.17	3.22	0.66	0.82	2.99	1.86	12.20	~~7.8~~	156.42
Zn	0.67	4.01	2.15	1.34	0.44	0.55	1.02	0.64	6.53	~~5~~	130.56
Mg	5.65	33.90	56.05	34.89	10.66	13.28	32.29	20.10	102.17	~~77.5~~	131.83
K	77.75	466.50	532.63	331.56	129.52	161.26	182.59	113.66	1072.98	~~700~~	153.28
Na	21.75	130.5	122.21	74.83	67.46	83.99	94.93	59.09	348.42	~~335~~	104.00
Cu	0.04	0.23	0.19	0.12	0.04	0.06	0.18	0.11	0.51	~~0.3~~	170.39

*Average weight about 8.3 kg. †Recommended volume of milk intake for a 6- to 9-month old infant. ‡The portion size is based on gastric capacity of an infant aged 6- to 9-month old (30 g/kg of body weight) divided by 4 to make up for breakfast (30 g × 8.3 kg = 249 g/4). §The portion size is based on gastric capacity of an infant aged 6- to 9-month old (30 g/kg of body weight) divided by 4 to make up for lunch and dinner (30 g × 8.3 kg = 249 g/4). ¶The portion size is based on gastric capacity of an infant aged 6- to 9-month old (30 g/kg of body weight) divided by 4 to make up for snacks (30 g × 8.3 kg = 249 g/4). Daily intake calculated by the sum of milk and non-milk intake.

of salt (Melo et al., 2008; Scientific Advisory Committee on Nutrition, 2003; Zand et al., 2012b). The later highlights an important issue in relation to micronutrient interactions. For instance, when considering bone health, high levels of magnesium can suppress PTH secretion and disturb calcium homeostasis and increase bone density. In addition, along with increasing intestinal absorption of calcium and phosphorus, vitamin D also enhances intestinal absorption of magnesium, whereas phosphate and calcium can reduce the absorption of magnesium (Ilich & Kerstetter, 2000).

On the other hand, increasing calcium consumption may negatively affect the absorption of iron, which may impact on the occurrence of iron deficiency anemia (Hallberg, 1998). Between 6 and 9 months, full term infants are at risk of iron deficiency anemia because of inadequate iron stores and therefore require iron from their diet (Domellöf, Braegger, Campoy, Colomb, Decsi, & Fewtrell, 2014). Studies have reported lower iron absorption in infants when iron supplements have been given with milk compared with water (Heinrich, Gabbe, Whang, Bender-Götze, & Schäfer, 1975) and juice (Abrams, O'brien, Wen, Liang, & Syuff, 1996). However, Dalton, Sargent, O'connor, Olmstead, and Klein (1997) found no effect of calcium and phosphorus supplementation on iron status or iron deficiency in full term infants fed iron fortified formula between 6 and 15 months. It is important to mention that products C, G, S3, and BF show high-iron content, which is due to fortification of these products as illustrated in Table 1. In addition, brands D and H also show a high iron content; these products are from an organic product range. The unfortified infant food products only contribute 20% of iron in comparison with their fortified counterparts for the meal intake. This may be important for parents when selecting appropriate meals for infants. Furthermore, although less clear, calcium is believed to also reduce zinc absorption. A reduction in iron and zinc absorption may cause impaired neurophysiological functions (Sandstead, 2000). Excessive iron and zinc intake may also have a counter-effect on copper (Sandström, 2001). Although iron intake in this study is below the NIH UL (40 mg/day), zinc on the other hand is above (5 mg/day). However, copper intake from this study is also above recommendations; at present, there is no UL set for copper (Trumbo, Yates, Schlicker, & Poos, 2001).

The extent to which the excess intakes observed in this study will affect the bioavailability is unknown, and knowledge of the mechanisms involved is relatively limited and needs further attention, especially during infancy (Rosado, 2003).

3.2 | Vitamin D

The concentrations of vitamin D in selected complementary infant foods tested in this study are presented in Table 8. The results were further subjected to two factor ANOVA without replication analysis. The calculated *F* value for the ANOVA within groups (between the replicates) showed no significant difference with *p* values calculated (vitamin D $p = .62$), which indicates the consistency of measurements.

The total daily intake of vitamin D, again based on the standardized menu proposed by Zand et al. (2012a), was 9.66 μg/day, which is 138% of the RNI set at 7 μg/day and illustrated in Table 9, which is below the UL of 38 ug/day set by NIH (Institute of Medicine, 2011). It is important to mention that 120% of the RNI was supplied by the fortified infant formula, with only 18% being provided by weaning foods. In situations where infant formula intake is compromised or reduced, as it does when the infant becomes older, vitamin D intake may become inadequate, as the majority of the vitamin D at 7–12 months is being supplied by the infant formula. Furthermore, food sources are relatively low in vitamin D, therefore, may not supply adequate vitamin D that an infant/toddler requires for optimal development and may possibly even become deficient.

In a study by Skinner et al. (1997), however, estimated dietary daily intake of Vitamin D for infants aged between 6 and 12 months was 6.6 ug/day, which is slightly below the RNI. The lower daily intake reported by Skinner et al. compared with the result in this particular study may be due to the inclusion of breast fed infants as breast milk is known to be lower in vitamin D compared with fortified infant formula.

The recent National Diet and Nutrition Survey highlighted that the mean intake of vitamin D from foods is well below the RNI of toddlers aged 1.5–3 years (Food Standards Agency, 2014). In addition, vitamin D concentrations in breast milk are much lower compared with fortified infant formula; therefore, if the vitamin D status of the

TABLE 8 Concentration of vitamin D3 in commercial infant foods (mean [*n* = 3] and CV, coefficient of variation)

	VD1		VD2		VD3		VD4	
	n (3)	CV	*n* (3)	CV	*n* (3)	CV	*n* (3)	CV
Vitamin D3 (μg/100 g)	0.496	.156	0.288	.055	0.400	.003	0.855	.059

TABLE 9 Total daily intake of vitamin D3 by an infant aged 7- to 12-month* old, based on the gastric capacity of an 8-month old and a standard feeding regime composed of commercial infant food products and infant formula milk in relation to the reference nutrient intake (RNI)

	Infant formula		Meals		Total daily intake [d]	RNI	% RNI
	100 ml	600 ml[†]	100 g	249 g[‡]	μg/day	μg/day	
Vitamin D (μg)	1.40	8.40	0.51	1.26	9.66	7.00	137.95

*Average weight about 8.3 kg. [†]Recommended volume of milk intake for a 6- to 9-month-old infant. [‡]The portion size is based on the gastric capacity of an infant aged 6- to 9-month old (30 g/kg of body weight) to make up for lunch and dinner (30 × 8.3 = 249 g), lunch/dinner = 249/2 = 124.5 g[d]. Daily intake is simply calculated by the sum of milk (b) and non-milk intake (c).

breastfeeding mother is low, then the infant may not be receiving an adequate supply of vitamin D. Furthermore, although breastfeeding mothers and infants are recommended to receive supplements of vitamin D, national surveys document that the majority are not following recommendations (McAndrew et al., 2012). This potential reduction in vitamin D after the first year of life, and potentially in breast fed infants, may have a detrimental effect on bone health as current recommendations are based on calcium absorption and bone health. It is also important to mention that vitamin D has also been implemented in the functioning of the immune system, and further knowledge into the role of vitamin D for immune functions needs to be further explored (Muehleisen & Gallo, 2013). Prolonged exclusive breastfeeding without vitamin D supplementation will also be important (Ahmed, Atiq, Iqbal, Khurshid, & Whittaker, 1995; Mughal, Salama, Greenaway, Laing, & Mawer, 1999).

3.3 | Estimated energy intake

The breakfast and snack infant food products have been shown to be a good source of micronutrients; however, it is also important to consider the contributions made on an energy level from these products. Based upon the nutritional labels provided by the manufacturer, the products have been assessed for their contribution to energy. For a 7- to 12-month-old infant, the EAR for energy is 687 kcal/day based on a diet of mixed feeding (Scientific Advisory Committee on Nutrition, 2011). In Table 1, S1, S2, and S3 are all biscuit based snack products, which contribute a 28% higher energy contribution compared with S4, which is a yogurt product. Breakfast and snacks contribute a total portion size of 62.25 g/day each, which equates to 39% and 30% of the EAR of energy for breakfast and snacks, respectively. Similarly, a portion size of 124.5 g of commercial ready-to-feed meals provides 14%, and 600 ml of infant formula provides 59% of the EAR for energy. Based on these observations, the total daily intake of energy will exceed the EAR by 42%, which identifies an important issue in relation to excess calorie intake and the risk of obesity. The diet and nutrition survey of infants and young children has shown that at least 75% of boys over 7 months and 78% of girls are above the 50th percentile for weight compared against the UK WHO growth standards (Department of Health, 2013), which emphasizes that parents must be aware of the energy contribution that infant foods contribute and select products, which provide good sources of micronutrients for optimal growth and development without over consumption of macronutrients (Gidding, Dennison, Birch, Daniels, Gilman, & Lichtenstein, 2006).

It is important to note that one of the limitations associated with this study is that it is unlikely to represent the actual amount of consumption that is ingested and retained by the infant as it does not take into consideration wastage and fails to take into account any contribution from breast milk or homemade foods.

4 | CONCLUSIONS

This feasibility study investigates the ratio of Ca:P in conjunction with vitamin D and other essential elements (Cu, Fe, K, Mg, Na, and Zn) in a range of commercial infant food products in the UK. In addition, as this study is the first to include consumption of infant snack products, the level of total calorie intake is also calculated in order to assess total daily energy intake.

The Ca:P ratio of the infant's diet based on the standard feeding regime used in this study equates to 1.49:1, which is within the recommended range of 1.1–2:1 recommended by European Society for Paediatric Gastroenterology Hepatology and Nutrition. However, the actual total daily intakes of calcium and phosphorus were 176% and 155% above the RNI, respectively. The implication of excess intake of micronutrients warrants further investigation for long-term health effects.

The total dietary intake of vitamin D3 was determined to be 9.61 µg/day, which is 137% higher than the RNI. However, 120% is contributed from fortified infant formula. As weaning foods are typically low in vitamin D unless they are fortified and breast milk concentrations are typically low, vitamin D deficiency may arise when infant formula consumption is reduced, which is the case after the first year of life.

Finally the estimated total energy intake, from consumption of the products tested herein, is estimated to contribute to a high-calorie intake with a possible impact on obesity.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs. Devyani Amin and Mrs. Atiya Raza for their support and assistance in this work.

SOURCE OF FUNDING

The work was funded by the corresponding author.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS

EL and NZ formulated the scientific ideas. EL and DW conducted the experiments. EL, NZ, and TC wrote the manuscript.

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How to cite this article: Loughrill, E., Wray, D., Christides, T., and Zand, N. (2016), Calcium to phosphorus ratio, essential elements and vitamin D content of infant foods in the UK: possible implications for bone health, *Maternal & Child Nutrition*, doi: 10.1111/mcn.12368

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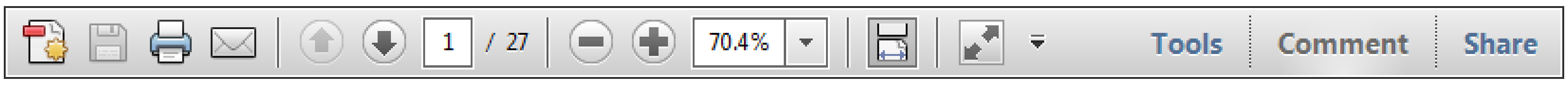
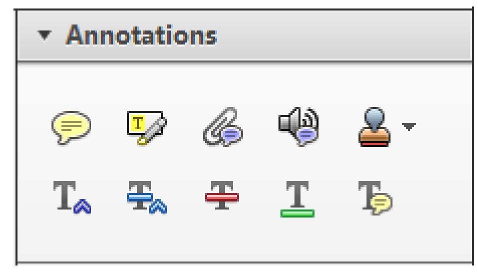
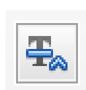


This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We've picked out some of these tools below:



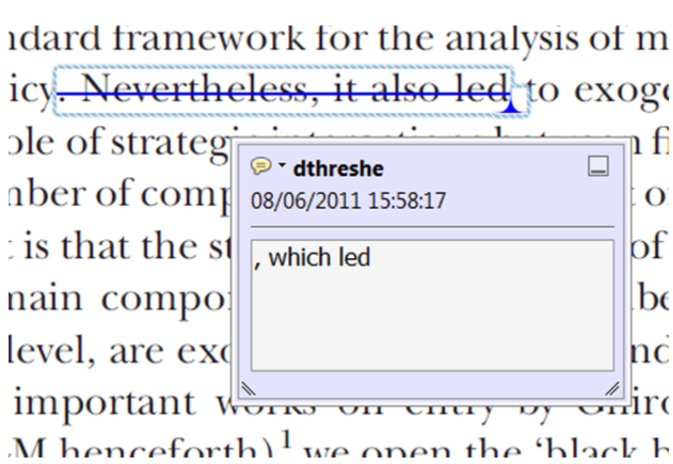
1. Replace (Ins) Tool – for replacing text.



Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the Replace (Ins) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

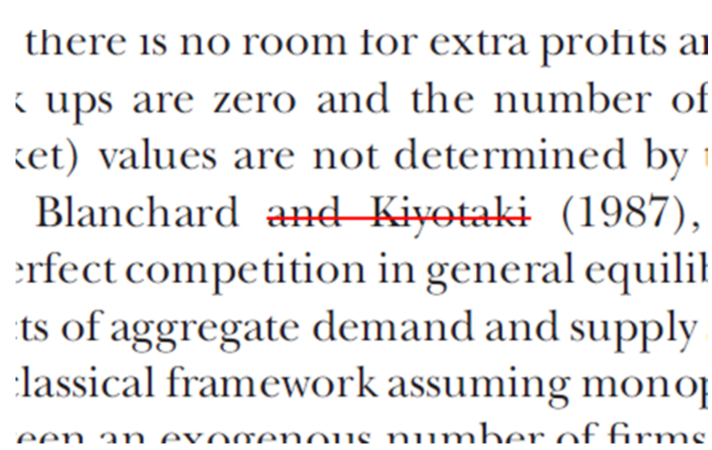


2. Strikethrough (Del) Tool – for deleting text.

Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the Strikethrough (Del) icon in the Annotations section.

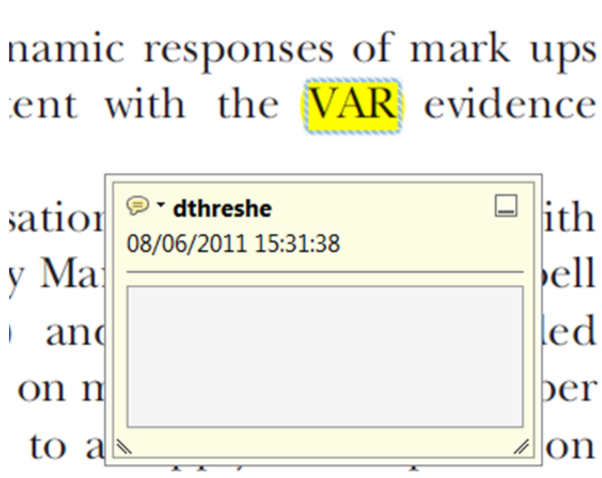


3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

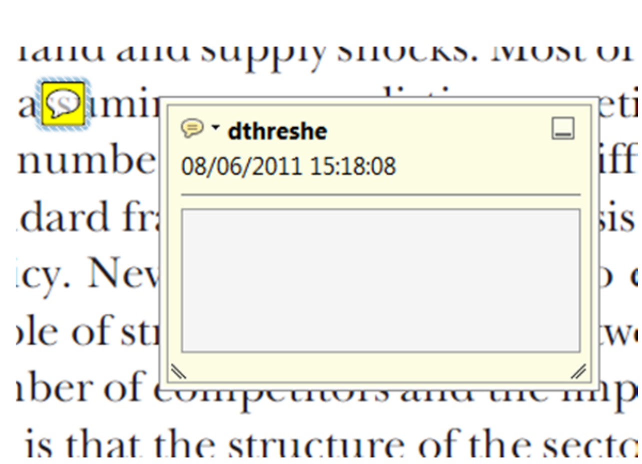


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

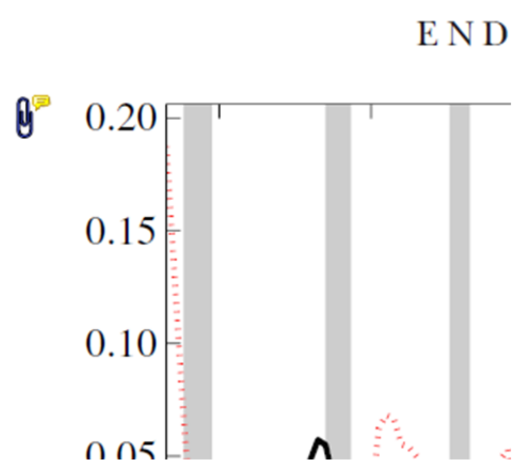


5. Attach File Tool – for inserting large amounts of text or replacement figures.

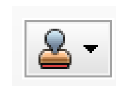
Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



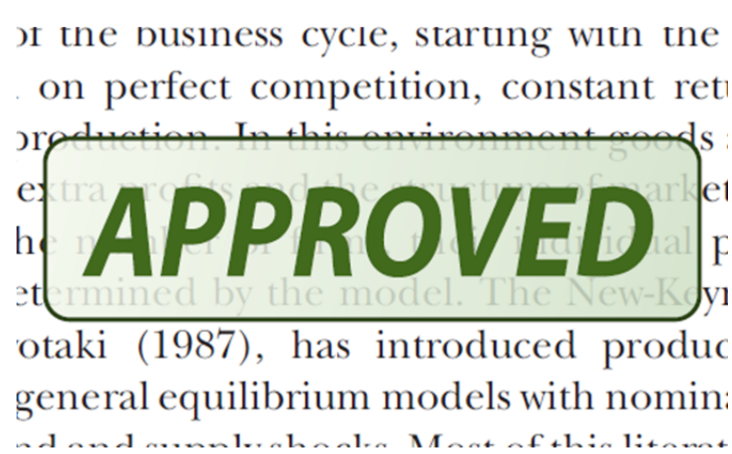
6. Add stamp Tool – for approving a proof if no corrections are required.

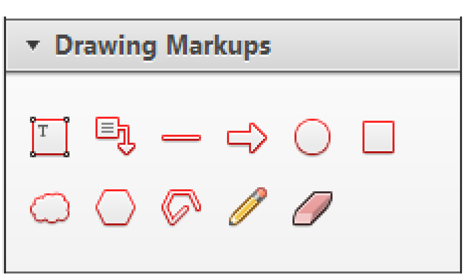


Inserts a selected stamp onto an appropriate place in the proof.

How to use it

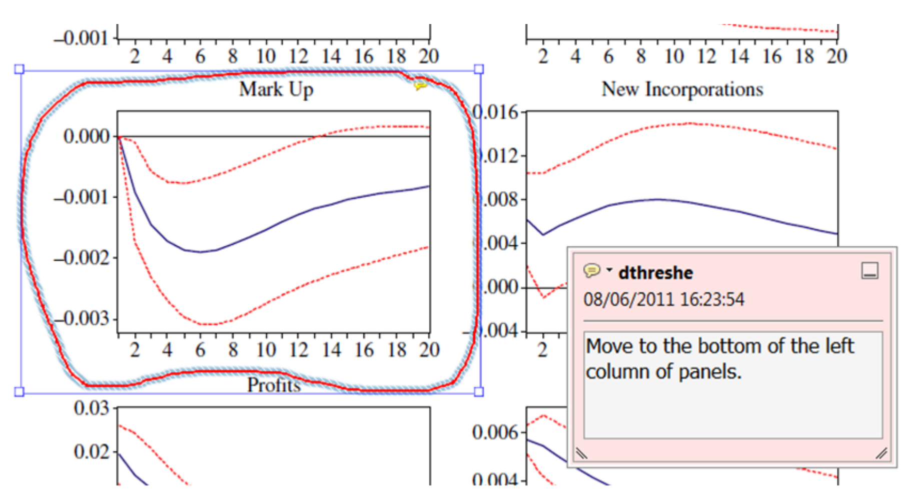
- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).





7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.

For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: