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An investigation into the fatty acid content of selected fish-based commercial infant foods in the UK and the impact of commonly practiced re-heating treatments used by parents for the preparation of infant formula milks

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ABSTRACT

The importance of dietary lipids during infancy is paramount for rapid growth and development. Linoleic acid (LA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) were quantified using RP-HPLC with charged aerosol detection in a range of complementary infant foods and formula milk. Total daily intake of fatty acids for infants aged 6–9 months was calculated based on the consumption of complementary infant foods and formula milk. Total daily intakes of ALA, AA and DHA were below, whereas LA was above the recommended intake. This provides scope for product optimisation, to improve the nutritive value of commercial infant food products. The impact of re-heating treatments by parents on fatty acid content of formula milk was investigated and statistically significant changes were observed. Furthermore, the transparency of the labelling information declared by the manufacturers was within recommendations despite a degree of significant variation.

1. Introduction

Traditionally lipids have been considered as a major contributor to dietary energy; however, recently the importance of the nature of dietary lipids ingested during infancy has been highlighted (Innis, 2007). Long-chain polyunsaturated fatty acids (LCPUFA) are thought to be a specific dietary requirement during infancy, due to a rapid rate of physiological and immunological development (Department of Health, 1991). Currently research has focused upon the LCPUFA, arachidonic acid (AA) and docosahexaenoic acid (DHA) for their essentiality and roles in the visual and brain development of infants (Simmer, Patole, & Rao, 2011). In addition, with the rise in the prevalence of allergies during recent years, a particular interest has also focused on *n*-3 LCPUFA, such as eicosapentaenoic acid (EPA) and DHA, for their possible protective roles in restricting the development of allergic disease (Calder & Miles, 2000).

Humans must obtain the essential fatty acids α-linolenic acid (ALA) 18:3 (*n*-3) and linoleic acid (LA) 18:2 (*n*-6) from dietary sources, as they lack Δ-15 and Δ-12 desaturase enzymes. ALA is then metabolised by Δ-6 desaturation, elongation and Δ-5 desaturation to form EPA 20:5 (*n*-3); LA is metabolised by the same enzymes to form AA 20:4 (*n*-6) (Innis, 2008). The synthesis of DHA 22:6 (*n*-3) however requires a second Δ-6 desaturase step and β oxidation. There is, however, much evidence to suggest that during infancy conversion of the essential fatty acid precursors (ALA and LA) into LCPUFA is insufficient due to the immaturity of the desaturase and elongase enzymes. Therefore, meeting the essential daily requirement through pre-formed dietary sources is paramount for infants (Makrides, Neumann, Jeffrey, Lien, & Gibson, 2000).

Several randomised controlled infant formula milk supplementation trials have demonstrated that infant feeding with DHA levels similar to the worldwide average (0.32% fatty acids) and AA levels higher than DHA provide visual and cognitive benefits to infants (Birch et al., 2010). Expert bodies now recommend that infants who do not receive breast milk should receive infant formula milk supplemented with DHA and AA (Hoffman, Boettcher, & Diersen-Schade, 2009).

In the UK exclusive breastfeeding is recommended for the first 6 months of an infant's life, with the only suitable substitute being infant formula milk. From the diet and nutrition survey of infants and young children (DNSIYC) conducted in 2011, 87–89% of infants

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aged 4–11 months are being given a type of infant formula milk. After 6 months complementary infant foods are recommended to be introduced. The DNSIYC has identified that 58% of children who are fed foods other than milk have eaten a commercial baby or toddler meal (Lennox, Sommerville, Ong, Henderson, & Allen, 2013). The results from the DNSIYC indicate that infant formula milk and commercial baby and toddler meals are major dietary contributors to an infant's nutritional intake. Therefore, these products need to be assessed for their nutritional adequacy. Furthermore there is currently no clear and complete analytical nutritional data available for commercial 'ready-to-feed' complementary infant foods in the UK. The nutritional database in the UK, McCance and Widdowson, contains limited data on the composition of complementary infant foods, in addition the analytical techniques and nutrient data contained may now be outdated. It is essential that food composition tables are regularly updated with the current foods available on the market, especially with the regular introduction of new foods to the market (Food Standards Agency, 2015).

The oxidation of lipids is induced by oxygen in the presence of initiators including heat, free radicals, light, photosensitising pigments and metal ions, through sequential free radical chain reaction mechanisms. Numerous compounds can be formed during these reactions, such as hydroperoxides and conjugated dienes (Martínez-Yusta, Goicoechea, & Guillén, 2014). Alterations in food products due to lipid oxidation can deteriorate the quality of the product both in terms of sensory and nutritional characteristics and can generate undesirable compounds, which can have effects on human health. In particular, unsaturated fatty acids are especially susceptible to oxidation (Santos-Fandila, Camino-Sánchez, & Zafra-Gómez, 2014). Infant formula milks are recommended to be served warm to the infant; meaning they will typically be subjected to a heat treatment prior to being given to the infant. Therefore it is important to assess any effect that a particular heat treatment has on the fatty acid content of infant formula milk.

The study herein investigates:

(a) The polyunsaturated fatty acid content of commercial 'ready-to-feed' complementary infant foods available on the UK market in order to evaluate whether they are nutritionally adequate in relation to recommendations for a 6–9 month old infant, also taking into consideration the consumption of infant formula milk.
(b) Assess whether commonly practiced re-heating treatments used for the preparation of infant formula milks by parents affect the polyunsaturated fatty acid content of infant formula milk.
(c) Examines the transparency of nutritional labels by comparing the analytically quantified concentrations of polyunsaturated fatty acids in infant formula milk to those provided on the packaging.
(d) Evaluate the content of polyunsaturated fatty acids in four infant formula milk brands available on the UK market based on their nutritional label.

2. Materials and methods

2.1. Chemicals

Linolenic acid, linoleic acid, arachidonic acid, docosahexaenoic acid and eicosapentaenoic acid, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) were obtained from Sigma–Aldrich (St. Louis, MO). HPLC-grade acetone, acetonitrile, tetrahydrofuran, formic acid, absolute ethanol, isopropanol, methanol and laboratory reagent grade chloroform and potassium hydroxide (KOH) were purchased from Fisher (Loughborough, UK).

2.2. Sample collection and analysis of complementary infant foods

Four fish-based commercial 'ready-to-feed' complementary infant food samples representative of the four leading brands in the UK (Statista, 2008a) were selected from leading supermarkets (Statista, 2008b) between June and July 2014. Three independent replicates were analysed from the same batch before the use by date of the product and within one month of purchase. The samples were stored unopened at room temperature, similar to their distribution and market environment.

Each of the food samples were mixed and homogenised using a domestic blender (Multiquick 300; Braun) and three independent replicates of 1 g (wet weight) were weighed prior to addition of 1.2 mL of chloroform/methanol (3:1, v/v) with 1 mg/mL BHT. The mixture was vortexed for 15 min followed by centrifugation at 35,500 RCF for 5 min to remove solids. One millilitre of the liquid phase extract was dissolved in 4 mL isopropanol/water (3:2, v/v) with 10 mg/L BHA and 1 mL 5 M KOH, which were then heated in an 80 °C water bath for 1 h with occasional stirring. After cooling at room temperature a 500 μL aliquot was removed and 25 μL of formic acid were added to neutralise the sample. A 50 μL aliquot of sample was then added to 50 μL of a standard solution (containing 0.5 mg/mL EPA, ALA, DHA and AA and 2.5 mg/mL LA) to prepare the spiked sample. Finally 15 μL of the spiked sample were injected into the HPLC-charged aerosol detector (CAD).

2.3. Sample collection and analysis of infant formula milk

A single brand of infant follow-on-formula milk was selected for the quantitative analysis in both the ready-to-feed (RTF) and the powdered form, in order to determine the impact of preparation methods on fatty acid content of formula milks. The selected infant formula milk was suitable for an infant aged 6+ months and available in supermarkets in the UK between June and July 2014. The selection of this particular brand was based on the inclusion of ALA, LA, DHA and AA content on the nutritional label of the infant formula milk, where other brands on the UK market did not provide such information. Three independent replicates were analysed from the same batch before the use by date of the product and within one month of purchase. The selected infant formula milk was stored unopened at room temperature, similar to its distribution and market environment.

Four different commonly practiced re-heating treatment methods of the selected infant formula milk were assessed, which are commonly practiced by parents and in accordance with the manufacturer's instructions including:

(a) Control RTF infant formula milk had no heat treatment applied prior to analysis.
(b) Heated RTF infant formula milk was prepared by placing the bottle of RTF infant formula milk in previously boiled water (15 min after boiling) as advised by the manufacturer. The infant formula milk reached a maximum temperature of 50 ± 0.5 °C, which was left to cool down to 37 °C prior to analysis.
(c) Microwaved RTF infant formula milk was prepared by microwaving 120 mL of RTF infant formula milk for 30 s, which reached a maximum temperature of 50 ± 0.5 °C, which was left to cool down to 37 °C prior to analysis.
(d) Powdered infant formula milk was prepared by reconstituting 6 scoops of powdered milk to 180 mL of previously boiled water at 88 ± 0.5 °C as advised by the manufacturer. The reconstituted infant formula milk reached a maximum temperature of 81 ± 0.5 °C and was cooled to 37 °C after being subjected to running cold water.