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Evidence of unmetabolised folic acid in cord blood of newborn and serum of 4-day-old infants

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Oral folic acid above certain threshold doses results in unmetabolised folic acid in serum. This raises a number of public health safety issues, principally the potential to mask pernicious anaemia; more recently the theoretical potential for high-dose folic acid to promote cancer has been highlighted. In this paper we set out to examine the appearance of unmetabolised folic acid both in cord blood from newborn full-term and premature infants and serum from 4-d-old infants post-formula feeding. Blood was collected from the umbilical cord of eleven infants in the delivery room immediately after birth. A follow-up serum sample (*n* 9) was collected 4 d later from infants post-formula feeding. We detected unmetabolised folic acid in cord blood from all infants at birth. In addition, unmetabolised folic acid was present in serum of seven infants post-formula feeding, six of which had increased from birth. Our results imply that infants in Ireland, which does not yet have mandatory fortification, could potentially have circulatory unmetabolised folic acid at the time of birth. We do not know if the presence of folic acid in cord blood will have any adverse consequences. However, if theoretical safety concerns are borne out by future research, the likelihood is that the longer the exposure the more likely the potential for harm. This would also be the case in infants exposed to unmetabolised folic acid as a result of formula feeding.

Folic acid: Cord blood: Infants: Ireland: Circulatory unmetabolised folic acid: Infant formula

The term 'folate' refers to the entire group of folate vitamin forms, i.e. the naturally occurring folypolyglutamates found in food, and folic acid. Folic acid is not found in nature, but is the synthetic form of the vitamin. Above certain threshold doses, oral folic acid in the form of synthetic pteroylmonoglutamic acid saturates the capacity of the intestinal mucosal cells to completely metabolise it into methylfolate, the natural blood form of the vitamin. This results in the appearance of unmetabolised, synthetic pteroylmonoglutamate in the circulation. This has been demonstrated in a young adult population (Sweeney *et al.* 2003) and also in the elderly (Kelly *et al.* 1997). The appearance of unmetabolised folic acid in circulation raises a number of safety concerns, the potential to mask pernicious anaemia (Weir & Scott, 1998) in an elderly population as well as potentially reducing the efficacy of anti-epileptic drugs (Chanarin *et al.* 1960) are well-documented concerns. More recently, the theoretical potential for folic acid to promote cancer has been highlighted (Charles *et al.* 2004; Kim, 2004). In countries where pregnant women consume folic acid either actively or passively there is the potential for unmetabolised folic acid to appear in the fetal circulation (Lucock *et al.* 1989; Kelly *et al.* 1997). It was recently hypothesised that early exposure of the fetus to excess folic acid may favour the genetic selection of the MTHFR 677T genotype which is associated with a range of developmental and degenerative conditions including CVD and Alzheimer's disease (Lucock & Yates, 2005). Some of these adverse effects may be partially explained by the anti-metabolic effect of synthetic folic acid.

In most developed countries many infants are fed formula food containing the synthetic form of the vitamin folic acid, not the natural 5-methytetrahydrofolate form, as it is readily degraded during heating (Johan & Magnus, 1980). The practice means that there is potential for unmetabolised folic acid to appear in the circulation of infants consuming formula milk. No previous studies investigating circulatory unmetabolised folic acid have been carried out in cord blood or in infants. The aim of the present study was to investigate levels of unmetabolised folic acid in cord blood of newborn infants whose mothers had been consuming a normal diet incorporating the standard commercially available folic acid-fortified foods in Ireland. It is noteworthy that mandatory fortification of a food staple such as is the case in the USA had not been implemented in Ireland at the time of the study and this remains the case today. In addition, we wanted to look at the effect on circulatory folic acid of feeding standard commercially available formula milk to 4-d-old infants.

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Methods

Recruitment

Ethical approval was obtained from the Coombe Woman's Hospital. Consent forms and parent information leaflets were issued to mothers who consented to their infant taking part in the study. We excluded babies who were breast-fed, babies who received supplemental feeding (enteral or parenteral), or infants with any metabolic or gut disorders, which might have interfered with the uptake of folic acid. We recruited full-term (*n* 8) and premature (*n* 3) babies. Two were twins born at 32 weeks and one was premature at 34 weeks.

Blood collection

A 1 ml blood sample was collected from the umbilical cord of each infant in the delivery room immediately after birth to provide a baseline serum folic acid concentration. A second serum sample was collected by venepuncture 4 d after the birth date at the time of the heel-prick test. This was collected approximately 90 min after their last formula feed to anticipate the T-max of folic acid (Kelly *et al.* 1997). All babies were on 3–4 h formula feeds. Eleven baseline samples and nine follow-up samples were collected due to refusal by two parents to allow the second sampling to take place after originally consenting to the study.

Dietary questionnaire

A questionnaire was issued to mothers. This included details on brand name of feed, number of feeds taken between birth and the second sampling, the amount taken at each feed and particularly the amount taken at the last feed prior to the second sampling. In addition, the mothers were asked about their use of folic acid supplements or other multi-vitamins at the time of cord-blood collection.

Formula feeds administered

Two standard formula feeds were routinely given to the babies in the present study at the discretion of the medical staff on the neonatal and post-natal wards. These included Cow and Gate Premium, which was fortified with folic acid at 10 μg/100 ml and SMA Gold Cap, which was fortified at 8 μg/100 ml (according to the nutrition label on these products). The type of feed consumed by each infant and the amount of folic acid consumed at their last feed prior to blood sampling are listed in Table 1.

Laboratory analysis

Serum folic acid. All serum samples were assayed for unmetabolised folic acid by HPLC/Sep-Pak/microbiological assay (Sweeney *et al.* 2003). This assay separates unmetabolised folic acid from other folates by HPLC. The separated folic acid pools were collected and concentrated by solid-phase extraction using C_{18} Sep-Pak cartridges. The methanol was then evaporated from the sample using a speed/Vac pump (Savant Instruments New York, USA). The dried sample was re-suspended in sodium ascorbate (0·5 %) buffer, plated out in triplicates of 100 μl on to a ninety-six-well microtitre plate and a *Lactobacillus casei* microbiological assay was performed (Molloy & Scott, 1997). Any triplicate sample not within 5 % standard deviation was repeated. The lower limit of accurately quantifiable detection of the assay is 0·3125 ng/ml. Concentrations below this level are detectable but may not be accurately quantifiable.

Formula folic acid. We measured the folic acid content of the formula feeds to validate that the babies were consuming the folic acid content stipulated on the formula feed label (Table 1). Total folate was extracted from the formula milk according to the procedure of Tamura (1998) and was measured by microbiological assay (Molloy & Scott, 1997). Formula folic acid levels were measured by HPLC/Sep-Pak/microbiological assay (Sweeney *et al.* 2003).

Statistical analysis

Statistical analysis was based on the Wilcoxon signed rank test and exact 95 % CI for a proportion.

Results

All of our infants (*n* 11) exhibited unmetabolised folic acid in cord blood (95 % CI 71·5, 100) (Table 2). Unmetabolised folic acid was also detected in seven out of the nine samples collected on day 4 of formula feeding. In six of these cases an increase from baseline occurred. The mean concentration of folic acid at birth was 0·185 μg/l (in the nine babies with a follow-up sample) and at day 4 post-formula feeding it was 0·468 μg/l (2·5 times higher).

We detected higher levels of folic acid in the formula milk than stated on the product label (Table 1). Cow and Gate Premium was fortified with folic acid at 10 μg/100 ml according to the product

Table 1. Folic acid (μg) content of formula feeds consumed according to product label versus our analysis

Subjects	Formula feed consumed	Folic acid in last feed as per product label (μg)	Folic acid in last feed as per our analysis (μg)
Baby 1 (full term)	Cow & Gate Premium	8	18·6
Baby 2 (full term)	SMA Gold Cap	Unknown	Unknown
Baby 3 (full term)	Cow & Gate Premium	4	9·3
Baby 4 (premature twin 1)	SMA Gold Cap	4	6·5
Baby 5 (premature twin 2)	SMA Gold Cap	4	6·5
Baby 6 (full term)	Cow & Gate Premium	8	18·6
Baby 7 (premature)	Cow & Gate Premium	8·5	19·76
Baby 8 (full term)	Cow & Gate Premium	5	11·625
Baby 9 (full term)	SMA Gold Cap	6	9·743
Baby 10 (full term)	SMA Gold Cap	6	9·743
Baby 11 (full term)	Cow & Gate Premium	Unknown	Unknown

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Table 2. Unmetabolised folic acid (μg/l) in serum of premature and full-term infants pre- and post-formula feeding

Subjects	Sample 1: at birth (from cord)	Sample 2: day 4 (venepuncture)
Baby 1 (full term)	0·08	1·31*
Baby 2 (full term)	0·47	No sample
Baby 3 (full term)	0·22	0·0
Baby 4 (premature twin 1)	0·22	0·31*
Baby 5 (premature twin 2)	0·17	0·54*
Baby 6 (full term)	0·31	0·18
Baby 7 (premature)	0·15	0·32*
Baby 8 (full term)	0·16	0·86*
Baby 9 (full term)	0·14	0·0
Baby 10 (full term)	0·22	0·70*
Baby 11 (full term)	0·43	No sample
Mean folic acid (μg/l) (inclusive of nine babies with follow-up sample only)	0·185	0·468

* Denotes an increase in unmetabolised folic acid concentrations from baseline.

label, however our analysis reported 23·25 μg/100 ml. SMA Gold Cap was fortified with folic acid at 8 μg/100 ml according to the product label, however our analysis reported 12·99 μg/100 ml.

Discussion

All of the infants sampled in the present study exhibited unmetabolised folic acid in their cord blood. This means that infants born in Ireland under the present conditions (in the absence of mandatory fortification) could potentially have unmetabolised folic acid in cord blood at birth. None of the mothers were consuming folic acid supplements. The levels of unmetabolised folic acid detected in the cord blood must therefore have arisen due to recent dietary intake of folic acid from commercially available fortified foods.

It has been previously documented that many serum constituents including water-soluble vitamins measured at birth in maternal and cord blood show higher concentrations in the cord than in the mother's blood (Berger, 1998). Unmetabolised folic acid has never previously been measured in cord blood. The presence of unmetabolised folic acid in cord blood may hold potential for harm in terms of adverse effects in the developing fetus, particularly if large doses are able to pass over the placenta barrier to the fetus. Adverse effects of large doses of folic acid have previously been demonstrated in a rat model where long-term high levels of folic acid supplementation resulted in lower birth weights and shorter vertex–coccyx length (Achon *et al.* 1999). It must be remembered, however, that other studies have shown a protective effect of folic acid supplementation in pregnancy and in some cancers (Thompson *et al.* 2001). Additionally long-term supplementation with folic acid has been demonstrated to reduce the risk of colorectal cancer (Giovannucci *et al.* 1998). The increase in folic acid concentrations after formula feeding in some of the infants is also noteworthy. The amounts of folic acid administered in the feeds were in the region of 4–10 μg per feed according to the product label. However, our assays showed that the levels actually consumed were higher than this. This may be a problem of overage like that reported in the USA (Quinlivan & Gregory, 2003). The feeds were administered every 3–5 h for only 4 d. The levels of folic acid in the serum increased over this time in some of the infants. If these levels continue to increase through childhood and adult life there is a possibility that individuals could be exposed to circulatory folic acid for their entire lives.

Modern obstetric practitioners advise against the consumption of pharmacological agents during early fetal development, yet mothers are advised to consume 400 μg supplements for the first 12 weeks of pregnancy. The beneficial effects of this in neural tube defect prevention are undisputed. It is not known whether unmetabolised folic acid is the active ingredient in the preventative mechanism but it seems unlikely as early observational studies (Hibbard & Smithells, 1965) illustrated the protective effect of a high folate status obtained from natural sources pre-dating fortification or supplementation. Should we expose the developing fetus to excess folic acid if this excess amount has no direct benefits? A balance must be struck between preventing neural tube defects in early pregnancy and megaloblastic anaemia in later pregnancy with exposure of the fetus to excess unmetabolised folic acid. Mandatory fortification has not yet been implemented in Ireland, however, an expert group (Food Safety Authority of Ireland, 2003) has recently recommended it. The Committee on Medical Aspects of Food and Nutrition Policy (2000) report also supports universal folic acid fortification of flour in the UK. Unmetabolised folic acid concentrations are likely to be much higher in infants currently exposed to mandatory fortification and as well as standard commercially available fortified foodstuffs as is the case in the USA. This paper illustrates the need for further debate on threshold doses necessary for maximising beneficial effects while minimising any adverse ones.

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