Fluoride retention in infants living in fluoridated and non-fluoridated areas: effects of weaning

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Abstract:

Limited knowledge is available on total fluoride exposure, excretion and retention in infants, despite the first year of human life being the critical period for dental development and the risk of dental fluorosis. This study aimed to investigate total daily fluoride intake (TDFI), excretion (TDFE) and retention (TDFR) in infants living in fluoridated and non-fluoridated water areas at pre- (<6 months-old) and post-weaning (6-12 months-old) stages of development. Healthy infants, aged 0-12 months, were recruited and their TDFI (mg/kgbw/day), from diet and toothpaste ingestion, was assessed over a 3-day period using a dietary-diary and toothbrushing-questionnaire. TDFE (mg/kgbw/day) was estimated by collecting 48h urine and faeces. TDFR (mg/kgbw/day) was estimated by subtracting TDFE from TDFI. Forty-seven infants completed the study; 16 at pre-weaning and 31 at post-weaning stages, with a mean age of 3.4 and 10.0 months, respectively. TDFI was lower in the non-fluoridated area (mean difference:-0.027; $p<0.001$) and at pre-weaning stage (mean difference:-0.027; $p=0.002$) but higher in formula-fed infants (mean difference:+0.037; $p<0.001$). TDFE was mainly affected by type of feeding with higher excretion in formula-fed infants (mean difference:+0.017; $p<0.001$). TDFR was lower in the non-fluoridated area (mean difference:-0.024; $p<0.001$) and at the pre-weaning stage (mean difference:-0.033; $p<0.001$) but higher in formula-fed infants (mean difference:+0.020; $p=0.001$). In conclusion, a relatively large proportion (65%) of fluoride intake is retained in the body (i.e. deposited in calcifying tissues) in weaned infants. This is an important consideration in fluoride-based prevention programmes with goals to maximise caries prevention while minimising dental fluorosis risk.

Introduction:

Fluoride (F) is mainly associated with calcified tissues in the body [(1)]. It stimulates bone cell proliferation and increases new mineral deposition in cancellous bone [(2)]. Topical F when applied to erupted teeth, for example through water fluoridation and toothbrushing with a fluoridated dentifrice can help reduce dental caries by almost 50% and is therefore a valuable public health measure to improve oral health [(2)]. Dental decay in children's primary and permanent teeth continues to be a major public health problem, with untreated decay being the most common disease affecting humans worldwide [(3)]. Decay experience is higher in lower socioeconomic groups: 83.4% of 5-year-olds in lower-middle income countries have tooth decay compared with 49% of 5-year-olds in higher income countries [(3)]. This high decay prevalence highlights the importance of primary F-based prevention programmes including fluoridated water, salt and milk. However, several studies[(4; 5)] have shown increased dental fluorosis (mainly mild) prevalence in both fluoridated and non-fluoridated communities, as a result of excessive systemic exposure to F from multiple sources. Dental fluorosis is visible clinically as a white or brown "mottling" of the tooth surface. The critical period for development of fluorosis in developing primary teeth is from 4 months *in utero* until 11 months of age. [(6)] Nonetheless, dental fluorosis in permanent maxillary central incisors is most likely to result from excessive F exposure during the first 4 years of life, with the first 12 months being most vulnerable period. [(7)]

In children, the tolerable upper intake level (UL) of 0.1 mg/kgbw/day has been suggested to minimise the risk of dental fluorosis.[(8,9)] Therefore, total daily F intake (TDFI) at both an individual and community level should be considered when setting guidelines for F use to maximise decay prevention and minimise dental fluorosis. Milk (breast- or formula-) is the main source of F intake in <6-month-olds. However, introducing complementary feeding (i.e.

weaning) and tooth-brushing, which usually starts at about 6 months of age, could have a marked impact on TDFI.[10]

Several factors such as diet composition, age and body size could alter the rate of F ingestion, absorption and retention [1; 11]. Therefore, it is important to quantify the body-retained F rather than only the absolute TDFI.

Knowledge about body F retention originates from studies of healthy adults or laboratory animals, despite the first year of human life being the critical period for dental development and the risk of dental fluorosis. This is mainly because of the practical difficulties of recording dietary F intake and collecting 24-hour urine and faeces samples from infants and young children to estimate TDFI, total daily F excretion (TDFE) and consequently body F retention. Therefore, the study aimed to measure TDFI, TDFE and total daily F retention (TDFR) in infants up to 12 months of age living in fluoridated and non-fluoridated water areas, at pre- (<6 months-old) and post-weaning (6-12 months-old) stages of development.

Methods:

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the School of Health and Social Care, Teesside University (#079/13), and the Faculty of Medical Sciences, Newcastle University (#00673/2013). Written informed consent was obtained from parental participants.

This observational study focused on parents of infants, aged 1-12 months, living in fluoridated and non-fluoridated water areas of north-east England. The parents were recruited between January 2014 and February 2016 through major universities, nurseries and 'baby-group-centres' in the selected areas. The participants were visited at their homes when

weights of infants were measured, using a calibrated digital baby scale (Learning Curve Brands Inc., UK), and samples/data were collected. The infants were included if they were healthy, had lived continuously in the selected areas, and were fed exclusively either breast-milk or formula-milk.

Estimation of TDFI: A food-diary was given to each parent with instructions on how to complete it on three consecutive days for their infant. Each parent was interviewed on the fourth day to ensure that all food/drink items consumed by their infant had been recorded. Samples of breast- and/or formula-milk, consumed by all infants, as well as samples of all home-made food and drink consumed by weaned infants during the three-day dietary assessment were collected for F analysis. Information on F concentration of commercially available baby food/drinks was taken from an existing F database.[12] Dietary F intake (µg/day) for each infant was estimated by multiplying the average intake of each item (g/day) by its F concentration (µg/g). In breast-fed infants, consumption of breast-milk was quantified from reported energy requirements, by age and gender,[13] and the average caloric density of human milk of 651 kcal/l.[14]

A parental oral hygiene questionnaire with interview was used to obtain information on the infant's toothbrushing habits and the frequency of cleaning/brushing, type and amount of any dentifrice used was recorded. The amount of F ingested through brushing was estimated from the weight and frequency of dentifrice use, assuming almost all dentifrice used in brushing is swallowed by an infant at this age.

No infant used F supplements, therefore the TDFI for each infant was calculated by combining F intake from diet and dentifrice use.

Estimation of TDFE: Parents were supplied with specially designed pads (Uricol, Sterisets, Newcastle-upon-Tyne, UK) for use inside the child's diaper (nappy) [15] and instructed on how to collect and store wet (urine) and/or soiled/wet (urine and faeces) pads/diapers over 48 hours. For wet pads/diapers, parents were asked to withdraw a 5-ml urine sample from the pad, using the plastic syringe provided, and transfer the sample to a labelled container. The collected samples were weighed to measure the weight of urine and/or faeces excreted. The faeces were removed from the pads using a disposable wooden spatula, transferred into a labelled bag and stored at -20˚C prior to F analysis.

To quantify the F content of each individual excrement (mg/sample), its measured F concentration (µg/g) was multiplied by the corresponding weight of excreta (i.e. either urine or combined urine and faeces; g). The TDFE for each infant was then computed by summing the calculated F content of individual excreta.

F analysis: Sample analysis were conducted from February 2016 to December 2017. F concentrations of home-prepared food and milk samples consumed by infants and excreted faeces were measured by the acid-diffusion method, with F assay of urine, non-milk based drink and water samples undertaken using a direct method with F-ion-selective electrode (Model 9409, Orion) and meter (Model 900A; Orion) after addition of TISAB-III, with the sample-TISAB ratio of 10:1 (v/v).[16]

Analytical quality control (AQC) was undertaken by i) conducting incurred sample re-analysis (ISR) and ii) using a certified reference material (CRM). ISR[17] was performed by re-analysing 5% of samples in separate runs on different days from when the analysis was performed. The percentage difference between the results was determined using the following formula: [(Repeat – Original) ÷ Mean] x 100[17]. In addition, a paired t-test was used to test for the difference between the original and re-analysed (repeat) data. A certified reference

‘urine fluoride’ sample (#PC-U-F1703, Institut National de Santé Publique du Québec, Canda) was used as the CRM and tested blind by the study technician.

Data analysis: TDFR (mg/day) was calculated by subtracting TDFE (mg/day) from TDFI (mg/day) for each infant and the relevant variables were normalised by body weight (mg/kgbw/day). Fractional F retention (FFR) was estimated as the ratio between TDFR and TDFI.

Statistical analysis:

Sample size: A power analysis was undertaken using R to estimate appropriate sample size based on results from a previous study of TDFI by Zohoori et al [(10)], which used 19 infants per group. It was estimated that 3.9 individuals per group would be needed for 80% power.

Analysis of variance (ANOVA) was used to evaluate the effects of weaning stage (pre- vs post-weaning), area of residency (fluoridated vs non-fluoridated) and type of feeding (breast- vs formula-milk) on TDFI, TDFE and TDFR. Initially the area of residency, weaning stage and type of feeding were used as predictors, plus all interaction terms; non-significant interaction terms were sequentially removed, along with main effects where necessary, until the best minimal model was identified. Mean and 95% confidence intervals of the differences were calculated for the terms in the minimal model. Effect sizes were calculated as η^2 [(18)] which are more useful than the more widely-used measure of Cohen's *d* in an ANOVA design such as our experiment, where feasibility of recruitment to the study was a limiting factor. η^2 can be interpreted as the proportion of total variability in the response variable that can be accounted by the explanatory variable [(19)]. Cohen[(20)] provided approximate η^2 benchmarks for 'small' (0.01), 'medium' (0.06) and 'large' (0.14 or greater) effect sizes. Since the effect size is basically a way of quantifying the absolute size of the difference between two groups,

the larger the effect size, the more reliable the difference is. A result is more likely to be biologically meaningful if it is both statistically significant and has a large effect size.

Long-term relationships between age (predictor) and the response variables; i) TDFI, ii) TDFE and iii) TDFR were evaluated with the aid of standard linear regressions and Pearson correlation coefficients.

Results:

Initially 77 parents expressed an interest to participate in the study, of whom 28 subsequently did not sign the consent form due to the extent of the required commitment from them (e.g. collection of urine/faeces samples). Two infants were excluded since they were receiving combined-feeding (breast and formula).

The majority (64%) of infants were males. Table 1 presents mean (SD) age and weight of the 47 infants, who completed the study, per group. The study managed to recruit more than 4 infants per group for all but two exposure combinations: un-weaned breast-fed infants living in a non-fluoridated area (n=2) and weaned formula-fed infants living in a non-fluoridated area (n=3). The overall mean age and weight of the 16 un-weaned infants was 3.4 (SD 1.1) months and 6.3 (SD 1.1) kg, respectively and of the 31 weaned infants, 10.0 (SD 1.2) months and 8.9 (SD 0.8) kg, respectively.

In total, 416 wet (urine) and 245 soiled/wet (urine and faces) diapers were collected from all infants; i.e. on average 7 diapers/day/child. In addition, 124 breast-milk and 138 water samples as well as 94 food diaries were collected.

Regarding the AQC, the mean ISR was 11% (SD 9%), well below the recommended ISR acceptance criteria (within 20% for small molecules and 30% for macromolecules).[17] There

was no statistically significant difference in F concentration between original and repeat analyses (mean difference: 0.011 mg/l; 95% confidence intervals: -0.019, +0.041 mg/l). The measured F concentrations of the original and repeat CRM samples were 0.232 and 0.235 mg/l, respectively, which were within the acceptable range (certified target value: 0.283 mg/l; and acceptable range: 0.182-0.384 mg/l).

Figure 1 shows weight-normalised TDFI, TDFE and TDFR for all infants by weaning stage, type of feeding, and area of residency. The mean TDFI of all infants was less than 0.1 mg/kgbw/day. TDFR was negative in the breast-fed infants living in fluoridated (-0.005 mg/kgbw/day) and non-fluoridated (-0.003 mg/kgbw/day) areas as well as in the un-weaned formula-fed infants living in the non-fluoridated area (-0.007 mg/kgbw/day), whereas the highest mean TDFR (0.061 mg/kgbw/day) was observed in the weaned formula-fed infants living in the fluoridated area. The best linear models (Table 2) indicated that TDFI was significantly higher in fluoridated areas ($p<0.001$), in formula-fed infants ($p<0.001$) and post-weaning ($p=0.002$). There was also a strong interaction between the area of residency and type of feeding, with TDFI being significantly higher ($p<0.001$) where infants were both formula-fed and residing in fluoridated areas. However the observed small, medium and large effect sizes for area of residency ($\eta^2 = 0.001$), weaning stage ($\eta^2 = 0.068$) and type of feeding ($\eta^2 = 0.308$), respectively, clearly imply that type of feeding was the main factor affecting TDFI while weaning stage was the second most important factor.

TDFE was significantly ($p<0.001$) lower in breast- compared to formula-fed infants (with a large effect size; $\eta^2 = 0.145$), and there was an interaction between weaning stage and type of feeding, with TDFE being significantly ($p<0.001$) lower in un-weaned breast-fed infants. Since the area of residency had no effect on TDFE, either as a main effect or any interaction term, it was omitted from the final model.

TDFR was significantly higher in fluoridated areas ($p<0.001$) and in weaned infants ($p<0.001$), but lower in breast-fed infants ($p=0.001$). There was a significant interaction between area of residency and type of feeding ($p<0.001$), with TDFR being lower than would otherwise be expected where infants were breast-fed and lived in non-fluoridated areas. The large effect sizes detected for both weaning stage ($\eta^2 = 0.168$) and type of feeding ($\eta^2 = 0.210$) denote the significance of these two factors in TDFR.

Mean body-retained F as a percentage of TDFI (i.e. FFR) for all infants is shown in Figure 2. At pre-weaning, the FFR was only positive in formula-fed infants living in the fluoridated area. The overall mean FFR for all 31 infants, at the post-weaning stage, was +65 (SE 3) %.

The linear relationships between age and weight-normalised TDFI, TDFE and TDFR for breast-fed infants are presented in Figure 3 and for formula-fed infants in Figure 4. In breast-fed infants (Figure 3), the statistically significant positive correlation between age and TDFI (mg/kgbw/day) was moderate ($\rho=+0.51$, $p=0.012$), but it was strong ($\rho=+0.63$, $p=0.001$) between age and TDFR. In formula-fed infants (Figure 4), there was a statistically significant positive moderate correlation between age and TDFI ($\rho=+0.41$, $p=0.044$) as well as between age and TDFR ($\rho=+0.56$, $p=0.004$).

Discussion:

Clinically, the affinity of F for bone and teeth is high and fluoroapatite crystals which form at the surface of bone or enamel are important for tooth enamel hardness and bone mineral matrix stability.[21] Uptake of F during the pre-eruptive stage of enamel formation, is dentally important due to the increased risk of dental fluorosis development if systemic ingestion of F is chronically excessive in early infancy. Therefore, for fluoridation policy decision-making

and informing caries prevention programmes, continued assessment of total F exposure in young children has been recommended.[22; 23]

The mean TDFI of all infants, in the present study, was below the UL of 0.1 mg/kgbw/day[8,9]. Moreover, the present study showed that in infants, the TDFI was overwhelmingly affected by type of feeding; whereas the effects of weaning stage and area of residency were moderate (see interaction terms in Table 2). At the pre-weaning stage, milk (either breast- or formula-) was the sole source of F intake for these infants. The mean TDFI of <6-month-olds exclusively breast-fed and living in both fluoridated and non-fluoridated areas was almost negligible (Figure 1; 0.005 and 0.001 mg/kgbw/day, respectively). Generally, the F concentration of breast-milk is very low (<0.02µg/ml) with no significant difference in breast-milk F concentration found between mothers living in fluoridated and non-fluoridated areas[24], due to the limited transfer of F from plasma to breast milk.[25] Breast-fed infants typically receive up to a maximum "dose" of 0.2% of the maternal F intake.[26] A considerable difference in F intake between exclusively breast- and formula-fed infants has also been reported for US 2-6-month-olds living in a fluoridated area with 1mgF/l in drinking water; 0.001 mg/kgbw/day in the 3 breast-fed vs 0.141 mg/kgbw/day in the 5 formula-fed infants.[27]

A significant increase in the weight-corrected TDFI with increasing age was observed in both breast-fed (Figure 3) and formula-fed (Figure 4) infants. This increase is likely to be due to introduction of weaning food/drinks containing F and post-weaning fluoridated dentifrice use (i.e. in 6-12-month-olds). A positive trend between TDFI and age has also been reported for infants aged 1-12 months, living in non-fluoridated areas in England.[10] However, an inverse relationship between age and TDFI has been reported for 1-12-month-old English infants[10] living in a fluoridated area (0.97mgF/l) and US (Iowa)[28] infants living in areas with F water

concentrations of <0.3 to 2.0 mg/l. The between-studies differences in the age-TDFI relationship could be due to differences in age, feeding pattern and oral hygiene habits of the studied infants. For example, in the UK study[10], more than 70% of infants were younger than 6 months with 10% being exclusively breast-fed, compared with 34% and 15%, respectively, in the present study.

Despite the significant increase in TDFI with age, the TDFE remained almost constant with increasing age (Figures 3 and 4). This finding can be explained by the low renal function found in infancy, even when the appropriate correction is made for the small body size.[29] After birth, maturation is still a process involving all organs including the kidneys. Effective renal plasma flow increases from 83 ml/min/1.73m^2 in full-term infants to an adult rate of 650 ml/min/1.73m^2 at approximately two years of age and Glomerular Filtration Rate increases from 40 ml/min/1.73m^2 at birth to normal adult values of 100-125 ml/min/1.73m^2 by two years of age.[30] Consequently, infants and toddlers have limited capacity for metabolism and elimination of different drugs and elements such as F from the body as their renal function only reaches full capacity by the age of two years.

The TDFE was largely influenced by type of feeding with significantly lower TDFE in the breast-fed- compared with formula-fed-infants (Figure 1). However, the effect of type of feeding on TDFE was moderated by the weaning stage (Table 2). Although lower TDFE in the breast-fed infants could be explained largely by their lower TDFI, as observed in the study, differences in the degree of F absorption between breast- and formula-fed infants should also be considered. The systemic bioavailability of F from different sources is influenced by presence or absence of other nutrients; e.g. aluminium, calcium, magnesium, and chloride increase faecal F excretion, whereas dietary fats improve F absorption.[1]

Breast-milk contains more fat but fewer minerals than infant-formula, therefore faecal F excretion (and consequently TDFR) would be expected to be lower in breast-fed infants.

The TDFR was largely influenced by weaning stage and type of feeding (Table 2). In un-weaned infants (<6-month-olds), those breast-fed living in both fluoridated- and non-fluoridated areas, as well as those formula-fed living in the non-fluoridated area, had a negative F retention (Figure 2) indicating a negative F balance (i.e. F excretion>F intake). A negative F balance has also been reported for five 2-6-month-old breast-fed US infants living in a fluoridated area.[27] F balance in infants can depend on plasma F concentration and the concurrent F concentration in the rapidly exchangeable F pool of bone; i.e. a fall in plasma F concentration results in a net migration of F from bone to plasma and *vice versa*. F can cross the placenta and accumulate in foetal calcified tissues.[31] However, when F intake of very young infants is lower than that needed to maintain the *in-utero* plasma F concentration (e.g. in breast-fed infants), mobilisation of F from calcified tissues into plasma occurs, which results in a F excretion which exceeds the levels of F intake (i.e. negative balance).[1] However, the study found (Figure 2) no statistically significant differences in the proportion of TDFI which was retained in the body of weaned infants (9-12-month-old); indicating the effect of weaning (i.e. eating other sources of food other than just milk) on FFR. For example, the bioavailability of fluoride from infant milk formula reconstituted with water is 65%, whereas, a mixed diet may reduce absorption of fluoride by 47%. [32; 33].

The mean FFR of 49% (Figure 2) for the 2-6-month-old formula-fed infants living in the fluoridated area was lower than the corresponding figure of 58% calculated for 2-6-month old formula-fed US infants living in a fluoridated area.[27] In addition, overall mean FFR in the thirty-one 9-12-month-olds (65%) was higher than the corresponding value of 50% reported

for five 9-12-month-old US infants living in a fluoridated area[34] but in agreement with the 65% reported for 212 children aged <7 years.[35]

Limitations

The possible limitations of this study are the sample size (though only in 2 of the 8 sub-groups), convenience sampling strategy and non-equally sized subgroups. Despite the prolonged recruitment efforts, the poor recruitment rate was mainly due to the high burden of collecting detailed exposure data over 3 days as well as samples of urine and faeces over 2 days from infants by parents. However, the number of infants in the present study (n=47) was substantially higher than those recruited in similar studies: '5 breast-fed, 5 bottled-fed infants' in a F balance study[27] and '2 breast-fed, 15 formula-fed infants (11 females, 6 males)' in a F pharmacokinetic study.[36]

The main limitation of this study is that urine and faeces were not collected separately due to ethical issues related to more invasive methods of urine and faeces collection, such as use of metabolic beds, needed to separate collection of these types of samples.[36] Therefore no conclusions can be drawn from this study about the effect of type of feeding (breast vs formula) on the degree of excretion of the ingested F (i.e. faecal excretion) or the absorbed F (i.e. urinary excretion).

Conclusion

The data showed that the type of feeding is the single most important factor in F intake, exertion and retention in infants, although the area of residency also has smaller, but significant impacts on F intake and retention. The data have confirmed that F intakes of exclusively breast-fed infants, irrespective of area of residency, as well as un-weaned formula-fed infants living in non-fluoridated areas are so low that they result in a negative F

balance (i.e. mobilisation of F from calcified tissues). However, in weaned infants, a relatively large proportion of F intake is retained in the body (i.e. deposited in calcifying tissues). It is important that when implementing F-based prevention programmes for young children that this is taken into consideration. The goals for these interventions should be to maximise caries prevention while minimising dental fluorosis risk. Current evidence is that the main caries preventive effects of F are due to its local topical effects at the tooth surface, while dental fluorosis results from chronic excessive systemic ingestion of F from different sources during crucial periods of tooth development. In view of this, recommendations from the American Dental Association (ADA)[37] such as; '*continue breastfeeding throughout the first year of life*' and '*use (infant) formula reconstituted with water that is either F-free or has low concentrations of F when the potential risk for enamel fluorosis is a concern*', remain key to harnessing the caries benefits of F while minimising risk of fluorosis.

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Conflicts of interest

The authors report no conflicts of interest. The views expressed in this paper are those of the authors and not those of the funding body.

Authorship

Prof FV Zohoori conceptualized and designed the study, supervised data collection, drafted the initial manuscript, and reviewed and revised the manuscript.

Dr N Omid collected data and samples, carried out the laboratory analyses of samples, and reviewed the manuscript.

Dr R Sanderson coordinated data analysis, and critically reviewed the manuscript for important intellectual content.

Dr RA Valentine co-supervised data collection and reviewed and revised the manuscript.

Prof A Maguire conceptualized and designed the study, co-supervised data collection and reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work

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Table 1. Infant data stratified by weaning stage, type of feeding and area of residency.

Infant	1-6-month-old infants					6-12-month-old infants					All infants (1-12 months)				
		Age (months)		Weight (kg)			Age (months)		Weight (kg)			Age (months)		Weight (kg)	
	No.	Mean	SD	Mean	SD	No.	Mean	SD	Mean	SD	No.	Mean	SD	Mean	SD
All breast- and formula-fed	**16**	**3.4**	**1.1**	**6.3**	**1.1**	**31**	**10.0**	**1.2**	**8.9**	**0.8**	**47**	**7.8**	**3.4**	**8.0**	**1.6**
Breast-fed, all	***7***	***3.8***	***1.0***	***6.4***	***1.6***	***16***	***1.02***	***1.2***	***8.7***	***0.8***	***23***	***8.3***	***3.2***	***8.0***	***1.5***
Breast-fed, FA	5	4.0	1.2	6.6	1.8	8	9.9	1.2	8.5	0.7	13	7.6	3.2	7.8	1.5
Breast-fed, NFA	2	3.4	0.5	5.9	0.9	8	10.5	1.1	9.0	0.9	10	9.1	3.2	8.4	1.6
Formula-fed, all	***9***	***3.1***	***1.2***	***6.2***	***0.8***	***15***	***9.8***	***1.2***	***9.2***	***0.8***	***24***	***7.3***	***3.5***	***8.0***	***1.7***
Formula-fed, FA	4	3.4	1.0	6.5	0.4	12	10.0	1.1	9.2	0.8	16	8.4	3.1	8.5	1.4
Formula-fed, NFA	5	2.8	1.4	6.0	0.9	3	8.8	1.3	9.1	1.0	8	5.1	3.3	7.1	1.9

FA, fluoridated area (Mean (SD) F concentration of tap water: 0.86 (0.23) mg/l); NFA, non-fluoridated area (Mean (SD) F concentration of tap water: 0.12 (0.09) mg/l).

Table 2. Summary of results of best sequential linear model, with weight-normalised total daily F intake, excretion and retention (as the response). The non-significant variables are not presented in the table.

Source	Differences between variables			Eta-squared
	Mean	95% CI	P value	(η^2)
Total daily F intake (mg/kgbw/day) (TDFI)				
Area of residency (NFA vs FA)	-0.027	-0.043, -0.012	<0.001	0.001
Weaning stage (Pre-W vs Post-W)	-0.027	-0.043, -0.011	0.002	0.068
Type of feeding (FF vs BF)	+0.037	+0.022, +0.052	<0.001	0.308
Area of residency * Type of feeding interaction:				0.087
'FA:FF' vs 'FA:BF'	+0.056	+0.030, +0.081	<0.001	
'FA:FF' vs 'NFA:BF'	+0.056	+0.028, +0.084	<0.001	
'NFA:FF' vs 'FA:FF'	-0.048	-0.078, -0.018	<0.001	
Total daily F excretion (mg/kgbw/day) (TDFE)				
Weaning stage (Pre-W vs Post-W)	+0.006	-0.001, +0.013	0.085	0.004
Type of feeding (FF vs BF)	+0.017	+0.011, +0.023	<0.001	0.145
Weaning stage * Type of feeding interaction				0.055
'Post-W: FF' vs 'Post-W:BF'	+0.013	+0.002, +0.023	0.010	
'Pre-W: FF' vs 'Post-W:BF'	+0.023	+0.011, +0.035	<0.001	
'Post-W: FF' vs 'Pre-W:BF'	+0.015	+0.002, +0.028	0.016	
'Pre-W: FF' vs 'Pre-W:BF'	+0.026	+0.012, +0.040	<0.001	
Total daily F retention (mg/kgbw/day) (TDFR)				
Area of residency (NFA vs FA)	-0.024	-0.036, -0.012	<0.001	0.0003
Weaning stage (Pre-W vs Post-W)	-0.033	-0.045, -0.020	<0.001	0.168
Type of feeding (FF vs BF)	+0.020	+0.008, 0.032	0.001	0.210
Area of residency * Type of feeding interaction				0.104
'FA: FF' vs 'FA: BF'	+0.036	+0.016, 0.056	<0.001	

‘FA: FF’ vs ‘NFA: BF’	+0.038	+0.016, +0.060	<0.001
‘NFA: FF’ vs ‘FA: FF’	-0.042	-0.066, -0.090	<0.001

Area of residency: FA, fluoridated area (Mean (SD) F concentration of tap water: 0.86 (0.23) mg/l); NFA, non-fluoridated area (Mean (SD) F concentration of tap water: 0.12 (0.09) mg/l).

Weaning Stage: Pre-W, pre-weaning stage; Post-W, post-weaning stage

Type of feeding; FF (formula-fed), BF (breast-fed)

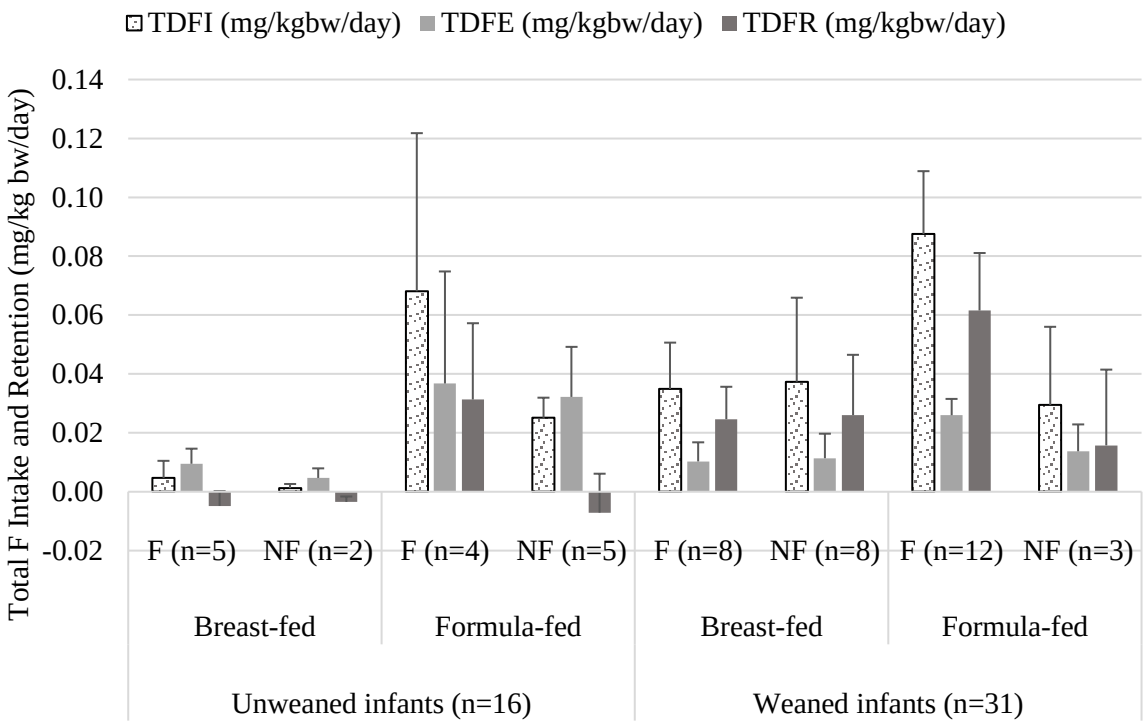

Figure 1. Mean (95% Confidence Interval) total F intake, excretion and retention (mg/kg bw/day) stratified by weaning stage (pre- vs post-weaning), type of feeding (breast- vs formula-milk), and area of residency (fluoridated (F) vs non-fluoridated (NF)) in infants younger than 12 months old.

Fluoridated area (0.86 ppmF); Non-fluoridated area (0.12 ppmF)

Un-weaned infant (<6 months old); weaned infant (9-12 months old)

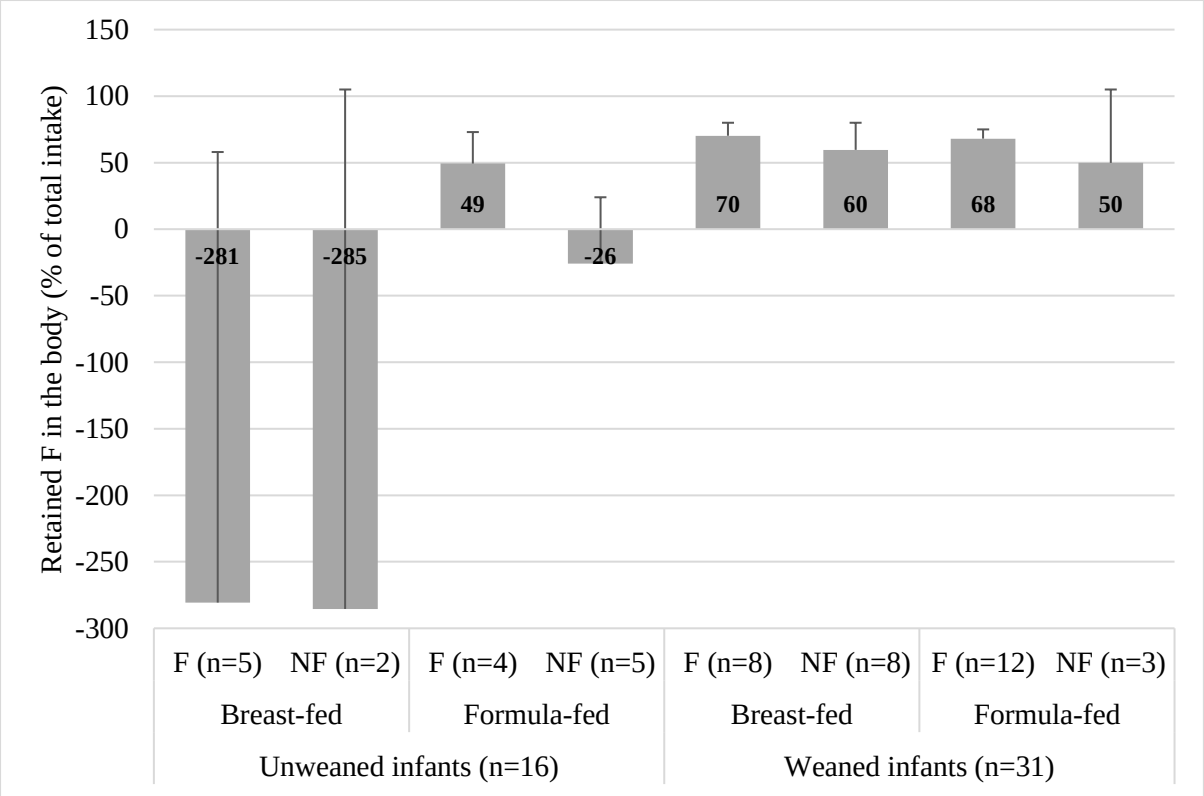

Figure 2. Mean (95% Confidence Interval) retained F in the body as a percentage of total F intake (FFR), stratified by weaning stage (pre- vs post-weaning), type of feeding (breast- vs formula-milk), and area of residency (fluoridated (F) vs non-fluoridated (NF)) in infants younger than 12 months old.

Fluoridated area (0.86 ppmF); Non-fluoridated area (0.12 ppmF)

Un-weaned infant (<6 months old); weaned infant (9-12 months old)

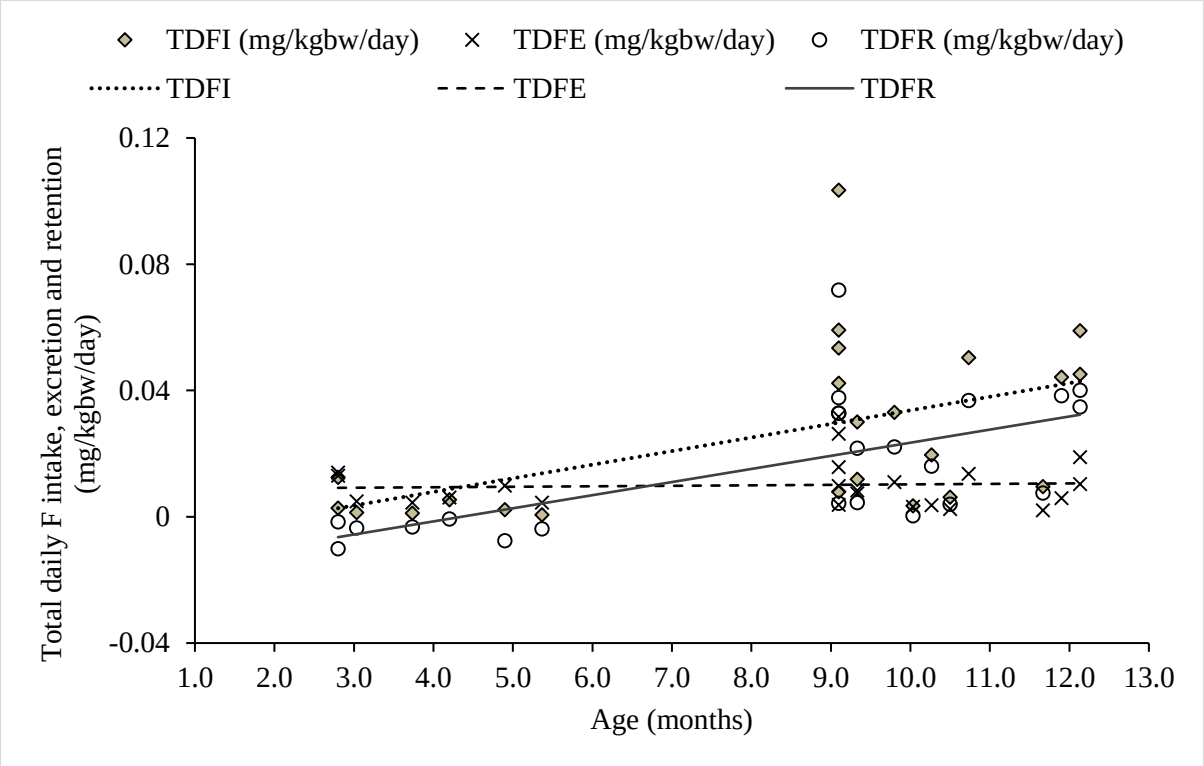

Figure 3. Relationship between age and weight-normalised - i) total daily F intake (TDFI), ii) total daily F excretion (TDFE) and; iii) total daily F retention (TDFR) in breast-fed infants (n=23).

TDFI (mg/kgbw/day) = -0.009 + 0.004 [age (months)]; (ρ = +0.51, p = 0.012)

TDFE (mg/kgbw/day) = 0.009 + 0 [age (months)]; (ρ = +0.06, p =0.774)

TDFR (µg/kgbw/day) = -0.018 + 0.004 [age (months)]; (ρ = +0.63, p = 0.001)

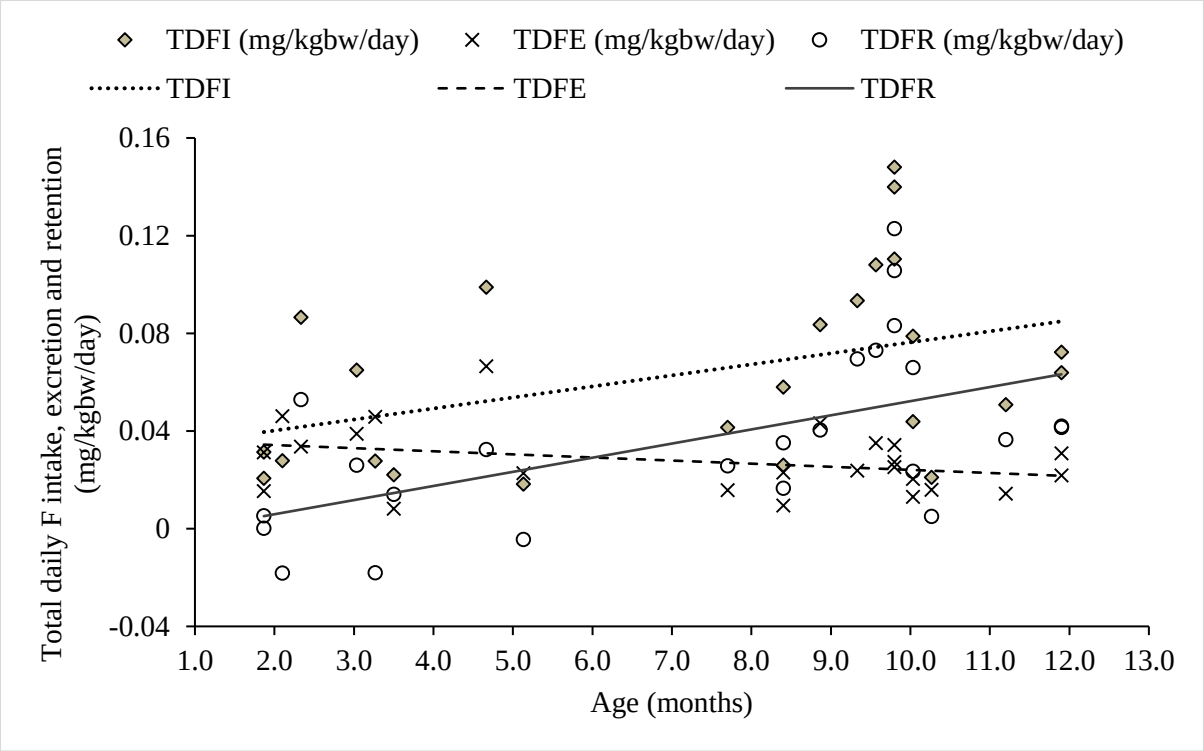

Figure 4. Relationship between age and weight-normalised - i) total daily F intake (TDFI), ii) total daily F excretion (TDFE) and; iii) total daily F retention (TDFR) in formula-fed infants (n=24).

TDFI (mg/kgbw/day) = -0.031 + 0.005 [age (months)]; (ρ = +0.41, p = 0.044)

TDFE (mg/kgbw/day) = 0.037 – 0.001 [age (months)]; (ρ = -0.32, p =0.122)

TDFR (mg/kgbw/day) = -0.006 + 0.006 [age (months)]; (ρ = +0.56, p = 0.004)