Fluoride intake and urinary fluoride excretion in 4- and 8-year-old children living in urban and rural areas of Southwest Nigeria.

Authors: O. Ibiyemi,[a,b] F.V. Zohoori,[c] R.A Valentine,[a] A. Maguire[a]

[a]Centre for Oral Health Research, School of Dental Sciences, Newcastle University, UK

[b]Institute of Health and Society, Newcastle University, UK

[c]School of Health and Social Care, Teesside University, UK

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Corresponding author:

Professor Anne Maguire

Centre for Oral Health Research, School of Dental Sciences

Newcastle University, Framlington Place

Newcastle upon Tyne,

NE2 4BW

UK

Email: anne.maguire@ncl.ac.uk

Tel: +44 (0) 1912088564

Fax: +44 (0) 1912085928

Abstract

Objectives: To estimate and compare total daily fluoride intake (TDFI), daily urinary fluoride excretion (DUFE), daily fluoride retention (DFR), fractional urinary fluoride excretion (FUFE) and fractional fluoride retention (FFR) in 4- and 8 year-old Nigerians and explore associations between these outcomes to improve understanding of fluoride metabolism..

Methods: Using a cross-sectional observational study, 72 four year-olds and 72 eight year-olds were recruited from nursery and primary schools (respectively) in lower and higher water F areas of urban and rural localities in Oyo State, south-west Nigeria. TDFI from diet and toothpaste ingestion was assessed using a validated Food Frequency Questionnaire and visual scale of toothpaste used during toothbrushing. DUFE was measured by collecting a 24-hour urine sample, FUFE estimated as the ratio between DUFE and TDFI, DFR estimated as TDFI-TDFE (where TDFE = DUFE + estimated faecal F excretion (i.e.TDFI x10%), and FFR was estimated as [(TDFI-DFR)/TDFI] x 100. Data were analysed using ANOVA with post-hoc tests and Students t tests and strengths of associations between key variables measured.

Results: Mean (SD) TDFI, DUFE, DFR, FUFE and FFR were 0.137 (0.169) mg/kg bw/d, 0.032 (0.027) mg/kg bw/d, 0.091 (0.147) mg/kg bw/d, 44% (44%) and 46% (44%), respectively for 4-year-olds. Corresponding values for 8-year-olds (n=63) were 0.106 (0.130) mg/kg bw/d, 0.022 (0.017) mg/kg bw/d, 0.073 (0.107) mg/kg bw/d, 36% (30%) and 54% (30%), respectively. Dietary contribution to TDFI was 79% and 75% (respectively), for 4- and 8-year-olds. Mean (SD) TDFI from toothpaste ingestion was 0.021 (0.013) mg/kg bw/d in 4-year-olds, 0.014 (0.010) mg/kg bw/d in 8-year-olds (p=0.002) but with no differences between areas. Differences in dietary F intake determined the main differences in F exposure between areas. The positive correlation between TDFI and DUFE was weak for 4 year-olds (r = +0.29) and strong for 8 year olds (r = +0.64). A strong positive correlation was observed between TDFI and DFR for both age groups: (r) = +0.98 for 4-year-olds and (r) = +0.99 for 8-year-olds.

Conclusion: Fluoride intake in these 4- and 8 year-old Nigerians was much higher than the "optimal range" of 0.05-0.07 mg/kg bw/d in rural, higher F water areas, with diet as the main contributor. F retention was similar in both age groups, with almost half of TDFI retained in the body. In terms of risk versus benefit for fluorosis and dental caries, this finding should be considered when mitigating against excessive fluoride exposure and planning F-based prevention.

Introduction

Excessive systemic exposure to fluoride (F) during tooth development can increase the risk of developing dental fluorosis [1]. Chronic excessive intake of F from birth to four years of age is considered to be a major contributor to the development of dental fluorosis in aesthetically important teeth. Excessive F exposure in older children can affect posterior teeth, although it is important to avoid too precise definitions of ages of greatest risk of fluorosis since there is increasing evidence that developing tooth germs may be vulnerable to excessive F exposure over an even longer period [2]. In view of the risk periods for dental fluorosis, it is important to understand and aim to control sources of excessive systemic F intake especially during periods of enamel formation.

A total daily F intake (TDFI) of 0.05-0.07 mg of F per kg of body weight (bw) has been considered as "optimal" during teeth mineralization to provide greatest resistance to dental caries and minimal risk of dental fluorosis. This guidance, although empirically-derived, has been adopted by many countries [3]. Information on TDFI is important for public health planners and health care professionals when planning community-based F therapy, as well as when mitigating against potential over-exposure to F [4]. Variations in F intakes across different communities arise from differences in dietary and toothbrushing habits as well as the local environmental conditions, such as source of water and its F concentration, temperature and consequently the volume of fluid intake. In addition, F intake within and between communities can vary considerably depending on an individual's diet as well as differences in the F concentration of the community's water supply used to prepare and cook meals.

The risk of dental fluorosis is positively correlated with body F burden or F retention, a value which can be derived by subtracting values for F excretion from F intake [5]. Factors such as the type of diet (eg. meat-based or vegetarian), urinary flow rate and renal tubular fluid pH can impact F retention by increasing or reducing the absorption and excretion of ingested F [6]. Due to the practical difficulties associated with collecting accurate data on F exposure in children, 24h urinary F excretion has been considered as a good biomarker of total daily F intake [7]. A comprehensive study using data for 212 children younger than 7 years, who consumed a 'westernised diet', concluded that reasonably good estimations of total F intake and retention can be obtained from daily urinary F excretion data at community levels [8]. However, the latter study suggested that further studies of F intake and excretion in children

older than 7 years are needed to explore how F intake and excretion data are related in older children. In addition, due to the diversity of diet among western- and eastern-based societies, more studies from developing countries would help to improve understanding of the relationship between type of diet (e.g. a vegetarian-based diet) and F excretion and retention. There are few data from sub-Saharan Africa, with the majority of research in these locations being observational studies of dental fluorosis [9-11] and skeletal fluorosis [12, 13]. In order to identify the primary sources of exposure to F and more fully understand the local risk factors for fluorosis, with a view to their mitigation in the longer-term, this study was designed to estimate TDFI, DUFE, TDFR, and the strength of the associations between these variables in 4 and 8 year-old Nigerian children living in naturally fluoridated areas.

Methods

The study was undertaken according to the guidelines in the Declaration of Helsinki [14]; the study protocol was approved by the Ethics Committees of Faculty of Medical Sciences, Newcastle University, UK and the University of Ibadan/University College Hospital, Nigeria.

This study represented Phase 2 of a larger study in which the prevalence of dental defects of enamel in Nigerian four and eight-year-old children was determined [15] and was undertaken between the end of the dry season (February) and mid-rainy season (July) in 2013.

Two Local Government Areas (LGAs), Ibadan North and Ibarapa Central, with a population size of approximately 306,795 and 102,979 respectively, were randomly selected from a list of all 33 LGAs in Oyo State (population size: 5.592 million), Nigeria [16]. Fluoride analysis of 124 water samples collected from common community ground water supplies (wells and boreholes) in rural and urban locations in these two LGAs was undertaken to identify 4 areas, namely urban higher F (0.80 – 1.0 ppm), rural higher F (2.0 – 3.0 ppm), urban lower F (0.04 - 0.07ppm) and rural lower F (0.06 - 0.07ppm) and these formed the setting in which the study was subsequently undertaken. The cluster sampling of 624 four (n=302) and eight (n=322) year-olds of both genders undertaken in randomly selected nursery and primary schools in Phase 1 of the overall project [15] was based on a power of 95% at an alpha level of 5% to determine a difference in mouth prevalence of DDE of 3% between areas with a non-completion rate of 30%. A 23% subsample (to allow for attrition) of these children was randomly selected and consented to participate in this present study. Inclusion criteria

included residence in the study area since birth, being healthy, with no history of metabolic disease or acid-base disturbance and not receiving a therapeutic diet.

Height (m) was measured without shoes using a portable stadiometer (DE56618903; ADE Germany) and weight (kg) was measured without heavy clothes and shoes using a portable digital scale (SOEHNLE, Slim Design Linea, Germany).

Participants' parents/guardians were interviewed about their child/ward's dietary and toothbrushing habits recorded using a previously validated, standardised, interviewer-administered semi-quantitative food frequency questionnaire (FFQ) [17] and a pictorial scale of the amount of toothpaste routinely used [18]. FFQs are used to measure food and drink intakes of individuals over a specific period of time, depending on the aim of the study (eg. over the previous 3-6 months, year or longer). The FFQ used was developed to include locally consumed diets and toothpastes and translated into local language (Yoruba), after which it was pre-tested among mothers with similar socio-demographic characteristics as the mothers of study participants and local language modified to ensure its reliability and validity. Samples of home-made food and drink consumed by the children plus drinking and cooking waters were collected from households and ready-to-consume samples of foods and drinks purchased from local shops as appropriate. A private interview was used to clarify the nature of food /drinks and obtain recipes if necessary. All food and drink samples collected were individually homogenized and stored frozen at -20ºC at the University of Ibadan, Nigeria before being transported on dry ice to Newcastle University, UK for appropriate F analysis. From a list of all toothpastes used by study participants, the same brands of toothpastes were purchased locally and stored at room temperature prior to F analysis.

Food and drink samples were pooled, based on their food/drink category, prior to F analysis. Waters and non-milk-based drinks were measured directly using a F-Ion-Selective-Electrode (F-ISE) after addition of TISAB III, while a hexamethyldisiloxane-facilitated diffusion method was used to measure the F concentration of food, milk-based drink and toothpaste samples [19]. A UK-based F database [20] was used to provide a best estimate of F content of any missing drink and food group samples since it reports F concentrations for similar foods and drinks cooked/prepared with similar water F concentrations. Each child's daily dietary F intake (DDFI), according to drink and food category, was estimated by multiplying the F concentration (mg/kg) of each category by the amount (kg) consumed per day and then

summing F intakes from each food and drink group to derive an estimate of F intake on a body weight basis (mg/kg bw/d).

The amount of F ingestion (mg/d) from toothpaste was estimated by multiplying the pictorially recorded amount of toothpaste used per brushing (mg) by its F concentration (mg/kg) and recorded frequency of daily use. This value was then derived on a bodyweight basis (as mg/kg bw/d) and multiplied by 41%: the mean % of toothpaste ingested per toothbrushing session reported for UK 4 to 6 year-olds [21] and Iranian 4-year-olds [22] since there is scarcity of global data for 8-year-olds and lack of any data from Nigeria. Total daily F intake (TDFI), in mg/d, was then estimated by adding DDFI and daily F intake from toothbrushing and their relative contributions determined. TDFI on a body weight basis (mg/kg bw/d), for each individual child, was then calculated by dividing TDFI by body weight.

To estimate daily urinary F excretion (DUFE), in mg/d, a 24-hour urine sample was collected once from each study participant using the method described by Zohoori and Rugg-Gunn, [22] its volume (ml) recorded and its F concentration (µg/ml) measured using a F-ISE and direct method [19]. Daily urinary F excretion was then estimated by multiplying urine volume and F concentration and the value normalised for individual bodyweight as mg/kg bw/d.

Total daily F excretion (TDFE) through urine and faeces was estimated by assuming that a fraction of 10% of TDFI is excreted through faeces and adding this to the DUFE [23, 24]. The TDFE value was then used to estimate total daily F retention (DFR) (mg/kg bw/d) using the following formula: DFR = TDFI-TDFE. In addition, to explore the body thresholds for excretion and retention of F in these 2 age groups, estimates were derived for fractional urinary F excretion (FUFE) and fractional F retention (FFR), based on the following equations:

FUFE (%) = [DUFE/TDFI] x100

and

FFR (%) = [TDFR/TDFI] x 100

The completeness of 24-hour urine samples was assessed by comparing urinary flow rate (ml/hour) with the World Health Organization (WHO) reference ranges for 4- (5-160 ml/hour) and 8- year-olds (9-300ml/hour) [7]. Participants with a urine flow rate outside this range were excluded from further analysis. The reliability and reproducibility of the F

analytical methods was examined by re-analysing 10% of samples and to confirm the validity of the analytical method, a known concentration F standard was added to another 10% of the samples, prior to re-analysis to measure F recovery.

Descriptive analysis was undertaken using SPSS version 21 (SPSS, Chicago, IL, USA) to derive mean (SD) values for each group. Statistically significant differences among areas were detected using ANOVA then investigated using a post-hoc test (Tukey) with statistical significance set at $\alpha < 0.05$. A student's t-test was used to compare the key variables between the two age groups. Pearson correlation was used to measure the strength of the associations between TDFI and; (i) DUFE; (ii) DFR, and; (iii) FFR. Based on Fisher's r-to-z transformation [25], the strength of the difference between the correlation coefficients obtained for the two age groups was assessed using the VassaStats website [26] for statistical computation.

Results

Sixty-four 4-year-olds and 68 eight-year-olds completed all aspects of the study and provided food/drink samples. Three 4-year-olds and five 8-year-olds did not meet the inclusion criterion of a urinary flow rate of 5ml/hour and 9 ml/hour respectively and they were excluded from further analysis. Overall, 48% of parents/legal guardians had received either no education (15% and 14% for parents of 4- and 8-year-olds respectively) or education to primary school level only (34% and 33% for parents of 4- and 8-year-olds respectively). The mean (SD) age, height and weight for 4 year-olds (n=61; 34 male, 27 female) was 4.5 (0.2) years, 1.0 (0.1) m and 15.5 (2.0) kg respectively; for 8 year-olds (n =64; 33 male, 31 female) it was 8.6 (0.3) years, 1.2 (0.1) m and 22.3 (3.2) kg.

Regarding the F assay methods used, the Incurred Sample Re-analysis for the 10% samples of water, food, drink and toothpaste showed that the F concentrations of the re-analysis were within 20% of the averaged concentrations between original and repeat measurements with no statistically significant difference in F concentration between test and re-test (mean difference was 0.007 mg/l). In addition, the mean recovery of F added to the samples was 94% with a range from 90% to 96%, representing an acceptable level of reliability and good validity for the F analysis method used.

Study participants consumed 31 food and drink types but 2 pooled samples (comprising steamed vegetables, and commercially available powdered milks added to tea, chocolate drinks etc.) from a low F area (actual drinking water 0.6 mg ppm F; actual cooking water 0.6 ppm F) were not provided by parents/guardians. These 2 missing food and drink samples represented 0.4% of the total weight of consumed food and drink and contributed 0.03% to the estimated daily dietary F intake from diet.

The F concentration of the common community ground water sources ranged from 0.06 - 3 ppm F and the 4 study areas were selected and categorised based on these F concentrations. Across the 4 study areas, the mean (SD) F concentrations of actual drinking and cooking waters consumed were 0.76 (0.90) and 0.68 (0.80) ppm F respectively, while the median (range) values for both waters were 0.40 (0.10 to 4.00) ppm F. No study participants took any F tablets or supplements; diet and inadvertent toothpaste ingestion were their only sources of F intake.

4-year-olds

Table 1 shows the estimated mean (SD) TDFI, DUFE, DFR, FUFE and FFR of 4 year-olds by area. The mean (SD) TDFI ranged from 0.050 (0.019) mg/kg bw/d in the urban lower F area to 0.385 (0.184) mg/kg bw/d in the rural higher F area. Diet was the predominant source of F, estimated as contributing an overall mean (SD) of 71% (19) of the TDFI (data not shown) with a range from 93% (6) in the rural higher F area to 54% (16) in the rural lower F area. Overall, 100% of the 4 year olds used toothpaste with a mean (SD) of 1.23(0.42) brushings per day and 0.52(0.28) g of toothpaste used per brushing (data not shown), with no differences between rural and urban communities.

Considering urinary F excretion, the overall mean (SD) 24h urine volume was 393 (197) mls (data not shown) and DUFE ranged from 0.021 (0.010) mg/kg bw/d in the rural lower F area to 0.053 (0.030) mg/kg bw/d in the rural higher F area (Table 1). The highest mean DFR (0.293 mg/kg bw/d) and lowest mean FUFE (18%) was estimated for the rural higher F area. The mean (SD) FFR ranged from 28% in the urban higher F area to 72% in the rural higher F area.

Comparison among the 4 study areas indicated no difference in total daily F intake from toothpaste ingestion, whereas F intake from diet and TDFI was higher (p<0.001) in the rural higher F area compared with the other 3 areas (Table 1). There was no difference in DUFE

between the urban and rural higher F areas, while it was lower in the two lower F areas than in the rural higher F area (p<0.01). In addition, DFR was higher in the rural higher F area than in the other 3 areas (p<0.001). A lower FUFE (p=0.029) and higher FFR (p=0.027) was found in the rural higher F area than in the urban higher F area, but neither FUFE nor FFR differed among the other areas.

8-year-olds

Data on the estimated mean (SD) TDFI, DUFE, DFR, FUFE and FFR of 8-year-olds by area is presented in Table 2. The mean (SD) TDFI ranged from 0.043 (0.016) mg/kg bw/d in the rural lower F area to 0.326 (0.128) mg/kg bw/d in the rural higher F area. Diet was the predominant source of F, estimated as contributing an overall mean (SD) of 74% (17) of the TDFI with a range from 94% (6) in the rural higher F area to 67% (16) in the urban higher F area. Overall, 98% of the 8 year olds used toothpaste with a mean (SD) of 1.08 (0.32) brushings per day and 0.57(0.27) g of toothpaste used per brushing (data not shown), with no differences between rural and urban communities.

The overall mean (SD) 24h urine volume was 618 (337) mls (data not shown). The mean (SD) DUFE showed a wide range from 0.012 (0.007) mg/kg bw/d in the rural lower F area to 0.044 (0.018) mg/kgbw/d in the rural higher F area (Table 2). The mean (SD) estimated DFR was highest in the rural higher F area (0.249 mg/kg bw/d) and lowest in urban lower F area (0.021 mg/kg bw/d). Consequently, the lowest estimated mean FUFE was 17% for the rural higher F area; the highest being found in the urban lower F area at 56%. As a result of this, the estimated mean (SD) FFR ranged from 34% in the urban lower F area to 73% in the rural higher F area.

Comparison among the 4 study areas showed no differences in total daily F intake from toothpaste ingestion, while F intake from diet was higher (p<0.001) in the rural higher F area than in the other 3 areas (Table 2). TDFI, UFE and DFR were higher in the rural higher F area than in the other 3 areas (p<0.001), while there was no difference in FUFE between urban and rural higher F areas and between the urban higher and lower F areas. FUFE was higher in the urban lower F than the rural higher F area (p=0.001) and the rural lower F area (p=0.017). However, FFR was lower in the urban lower F than the rural higher F (p=0.001) and rural lower F area (p=0.021).

Comparison between 4- and 8-year-olds

Table 3 presents means and 95% confidence intervals for differences in TDFI, DUFE, DFR, FUFE, FFR and urine volume between 4- and 8-year-olds. Total daily F intake from toothpaste ingestion was higher (p =0.002) in 4-year-olds (0.021 mg/kg bw/d) than in 8-year-olds (0.014 mg/kg bw/d). However, there was no difference in TDFI between the two age groups. Although there was no difference in urine volume, adjusted for body weight, between 4- and 8-year-olds, the DUFE was higher (p=0.013) in 4-year-olds (0.032 mg/kg bw/d) than in 8-year-olds (0.022 mg/kg bw/d). There were no differences in FUFE or FFR between the two age groups.

Relationships between the key variables

Figure 1 illustrates the relationships between TDFI and DUFE for both age groups. The slope and intercept of the linear relationship were 0.046 and 0.026 (respectively) for 4-year-olds and 0.085 and 0.013 for 8-year-olds. The positive correlation between TDFI (mg/kg bw/d) and DUFE (mg/kg bw/d) was weak for 4-year-olds (r = +0.29), but strong for 8-year-olds (r = +0.64). There was a statistically significant difference (p=0.012) in the correlation coefficient estimated for 4 and 8 year-olds.

The linear relationships between TDFI and DFR for both age groups are shown in Figure 2. A very strong positive correlation between TDFI (mg/kg bw/d) and DFR (mg/kg bw/d) was seen for both age groups: 4-year-olds (r = +0.98), 8-year-olds (r = +0.99). There was no statistically significant difference (p=0.06) in the correlation coefficient estimated for 4 and 8 year-olds. Although the intercept of the linear relationship differed between the two age groups (-0.026 in 4-year-olds and -0.013 in 8-year-olds), the slopes were very similar (0.854 and 0.815, respectively).

Figure 3 presents the association between estimated FFR with TDFI for both age groups. The FFR increased with increasing TDFI and plateaued at TDFI values greater than approximately 0.1 mgF/kg bw/d for both age groups.

Discussion

This is the first report of estimated F intake and excretion in children in Nigeria. In addition, to the best of our knowledge, this paper is the first to report the relationship between total F intake (from diet and toothpaste ingestion) and urinary excretion in 8-year-olds. A comparison of these F metabolism variables between two age groups living under similar

environmental conditions is also unique. When adjusted for body weight, the study found higher urinary F excretion in 4-year-olds compared with 8-year-olds, despite no difference in total daily F intake between the two age groups, but no difference in F retention. These changes in the pattern of F intake and excretion seen as a child develops from their primary to mixed dentition stages may be relevant when seeking to quantify dental fluorosis risk in populations.

Although the range of F concentrations of community ground water supplies sampled was 0.03 to 3.0 ppm F, the drinking and cooking waters actually consumed by participants were < 0.01 to 4.0 ppm F; within the range of 0.03-6.7 ppm [27] previously reported in Nigeria.

The F concentrations of actual drinking and cooking waters consumed varied slightly from those of the local community water supplies which had been collected from shallow wells and aquifers and used to categorise study locations into high and low F water areas. F concentrations of shallow wells can show high variability, some of which is due to seasonal differences with lower F concentrations found during rainy seasons. The actual drinking and cooking waters consumed showed less variation in F concentration between areas when analysed for fluoride. This was primarily because participants' individual drinking waters in particular, were less likely to have been obtained from the community water supply, but rather were commercially purchased waters, sold in sachets; a common practise in Nigeria to try and optimise drinking water quality. As a result, children in three of the four areas were exposed to fairly similar drinking water F concentrations.

Diet and toothpaste ingestion were the primary sources of F intake, with diet being the major component of TDFI for both age groups (Table 3). The 71% contribution of diet to TDFI, for the Nigerian 4-year-olds corresponds to the 70% reported for US children aged 4 years [28]. There are no data on TDFI in 8-year-olds, to compare our results with, however the literature shows a wide variation in the contribution of diet to TDFI ranging from 88% for 6-year-olds in Iowa [28] to 31% for 4–5-year-olds in Puerto Rico [29]. Differences in children's age, dietary habits and pattern as well as methods of data collection could account for the differences in dietary contribution to TDFI seen in these studies.

In terms of the type and constituents of diet seen, solid food components most commonly comprised starchy staple foods cooked with water for prolonged periods, which made a substantial contribution to F intake, particularly in the higher F rural area. A limitation of this study was that fluoride intake from toothpaste was determined visually by parents'

questionnaire responses to diagrams depicting the amount of toothpaste routinely used. The estimated 41% of dispensed toothpaste ingested was based on that reported for 4-6 year-olds in the UK [21] and Iranian 4-year-olds [22]. However, there was no evidence to suggest that this was any different in Nigeria where toothpaste use is widespread. This approach to estimation of toothpaste ingestion can be useful for informing epidemiological studies involving large numbers of people where individual toothbrushing behaviours cannot be observed directly and where estimates are required. Nevertheless, differences in quantities and F contents of toothpastes used and in toothbrushing habits can impact comparisons with other studies and therefore any extrapolation should be cautious.

The contribution of diet to TDFI (74%) for Nigerian 8-year-olds was slightly higher than for 4-year-olds, reflecting the lower contribution through toothpaste ingestion for the older age group. Children younger than 6 years are less able to fully control their swallowing reflex resulting in unintentional swallowing of more toothpaste compared with older children [30].

The present study demonstrated the impact of F concentration of water on TDFI and consequently UFE, TDFR, FUFE and FFR (Tables 1-3). In 4-year-olds who consumed drinking and cooking waters with a median F concentration of ≤ 0.07 ppm (i.e. urban higher F area and urban and rural lower F areas) (Table 1), the mean TDFI was within the 'optimum' range of 0.05-0.07 mgF/kg bw/d for maximum benefit in terms of caries reduction with minimised risk of dental fluorosis. However, for those four year olds in the rural higher F area the mean TDFI was 0.385 mg/kg bw/d, well above the UL of 0.1 mgF/kg bw/d, and potentially putting the children at greater risk of dental fluorosis. The same trend was also observed for 8-year-olds (Table 2); a TDFI range of 0.043-0.057 mg/kg bw/d for those children receiving drinking and cooking waters with median F concentration of ≤ 0.5 ppm versus a TDFI of 0.326 mg/kg bw/d for those receiving drinking and cooking waters with a median (range) F concentration of 0.3 (≤ 3.0) and 0.47 (≤ 3.0) ppm.

In the present study, the overall mean TDFI of 4-year-olds (0.137 mg/kg bw/d) was only slightly higher than 8-year-olds (0.106 mg/kg bw/d), whereas the mean DUFE was statistically significantly higher in 4-year-olds (Table 3). The estimated intercepts, presented in Figure 1, clearly indicate that in the absence of any F exposure, the DUFE for 4-year-old children was twice that of the 8-year-olds (0.026 mg/kg bw/d for 4-year-olds vs 0.013 mg/kg bw/d for 8-year-olds) which could be explained by the type/form of ingested F and its bioavailability. In general, the bioavailability of F from toothpaste (either NaF or SMFP) is

higher than that from a mixed diet. The amount of F intake from toothpaste ingestion in 4-year-olds (0.021 mg/kg bw/d) was higher than that in 8-year-olds (0.014 mg/kg bw/d) (Table 3), therefore, the amount of absorbed F would have been higher in 4-year-olds compared with 8-year-olds due to the higher bioavailability of the F from toothpaste. This indicates that F bioavailability is a highly relevant co-factor in body F burden when evaluating risk of dental fluorosis, alongside the actual amount of fluoride intake.

Physical activity/sedentary behaviour and/or skeletal development stage impacts DUFE, with renal clearance of F declining with increasing physical activity [31]. A recent study in South Africa, reported an increase in activity level with age; with 9-11 year-old boys and 12-14 year-old girls more physically and aerobically active than boys and girls aged 5-6 years [32]. The significantly lower DUFE in 8-year-olds in comparison with 4-year-olds, observed in the present study, could therefore relate to the effect of a higher level of physical activity on renal F clearance in the older children.

Greater rates of F uptake into newly formed bones (i.e. F retention) occurs during periods of rapid growth [6]. Daily skeletal gains of calcium with age seen from birth to puberty follow a 'V' shape with the peak gains being during the first months of life and then again during the adolescent growth spurt, whilst the lowest gain is around 3-4 years of age [33]. Although interpretation should be cautious in view of different populations and methods used, when the FFR data (% F retention) were plotted against age (Figure 4), for 8-year-olds together with similar but limited data in the literature for infants [24], 3-year-olds [34], 4-6 year-olds [35], 7-year-olds [36] and 11-14 year olds [37], the resultant graph also shows a similar 'V' shape for fluoride.

The observed overall mean FFR of 46% for 4 year-olds was lower than the 55% of TDFI estimated to be retained by pre-school children [8] but higher than the 11% and 15% reported for 4 year-old Iranian [22] and 3-4 year-old US [38] children respectively. In contrast, for 8 year-olds, the estimated FFR at 54% was similar to the 54% reported among 4-5 year-old Chilean children [23].

The present study showed a positive linear relationship between TDFI and DFR (Figure 2) for both age groups, with similar slopes; 0.854 and 0.815 for 4- and 8-year-olds, respectively, which implies a higher F retention with increasing F intake. However, Figure 3 suggests that the FFR reaches a limiting constant proportion of 80% above a TDFI of approximately 0.1 mg/kg bw/d, a value similar to the suggested Tolerable Upper Intake Level (UL) for F [3]. This constant proportion of 80% for FFR seen in both age groups above this TDFI threshold is

higher than the 50% suggested by Ekstrand for infants aged 2-5 months [24] and the 55% found by Villa et al [8] in a broader age group of children, and should be considered further when assessing any increased risk of fluorosis from excessive F accumulation in hard tissues due to the higher levels of F exposure seen in some of these Nigerian children.

In the present study it was useful to be able to compare the FUFE between these two age groups subjected to similar environmental influences including diet. The lower FUFE seen in the older children is consistent with results from other studies of differing age groups in the same environment, in which older children retain a greater % of their F intake up to threshold levels [8]. The present study eliminated some of the differences in confounding variables seen in different populations, providing a clearer picture of the difference in FUFE (and therefore body F retention) seen by age.

In 4-year-olds, there was no significant difference in FUFE among the three areas in which the median (range) of drinking and cooking waters was 0.07 ($\leq$ 0.6) ppm F but FUFE in these 3 areas was significantly higher than in the rural higher F area with 0.3 ($\leq$ 4.0) ppm F waters. However, interestingly, in 8-year-olds, despite no difference in TDFI and DUFE among the three areas with a median (range) water F of 0.08 ($\leq$ 0.5) ppm F, the FUFE was significantly higher in the urban lower F- (median (range); $\leq$ 0.5 ($\leq$ 1.0) ppm F) than in the rural lower F- (median (range); $\leq$ 0.08 ($\leq$ 0.5) ppm F) and rural higher F (median (range); $\leq$ 0.47 ($\leq$ 3.0) ppm F) areas. These findings may be explained by possible differences in dietary composition and patterns between rural and urban areas as well as between 4- and 8-year-olds, all of which affect F absorption and excretion. For example, an increase in the proportion of TDFI excreted in the urine has been shown for groups consuming rice-based diets compared with sorghum-based diets [39]. It has also been suggested that high dietary concentrations of certain cations such as calcium in milk can reduce the extent of F absorption [40]. Furthermore, F absorption may increase when diets rich in protein and fats are consumed since they reduce the rate of gastric emptying, while diets which are primarily vegetarian alkalinise the urine resulting in decreased reabsorption of F [41]. In the present study, no comparisons on dietary composition and habits were made between rural and urban areas or between 4- and 8-year-olds. The effect of dietary composition on F absorption and excretion merits further investigation, not only in Nigeria, but also globally.

The overall mean FUFE of the Nigerian 4-year-olds was 44%; very close to the 42% reported for UK 4-year-olds [35]. To the best of our knowledge, there are no FUFE data in the literature

for 8-year-olds. However, the overall mean FUFE of the Nigerian 8-year-olds, (36%) was similar to the FUFE of 35% reported for Chilean 11-14-year-olds [37].

The present study found a positive linear relationship between TDFI and DUFE (Figure 1) for both age groups, however, their slopes of the correlation differed (0.046 and 0.085 for 4- and 8-year-olds, respectively). This finding provides further evidence that DUFE can be used to estimate TDFI; similarly to the study by Villa and co-workers [8], in which the relationship between UFE and TDFI was examined using previously published data on F intake and excretion in children younger than 7 years old, and showed that, on average, 35% of TDFI was excreted in the urine. The findings of the present study supplement this latter study, filling gaps in the dataset and suggest that: (a) F intake and excretion data of 8-year-olds fits well within the distribution of corresponding data for younger age groups; and (b) similarities exist in F intake and excretion data, collected from developing and developed countries, mainly due to globalisation of the food system and disappearance of food traditions in developing countries in recent years.

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Legends for Tables and Figures.

Table 1. Estimated Mean (SD) fluoride intake (mg/kg bw/d), urinary fluoride excretion (mg/kg bw/d), fluoride retention (mg/kg bw/d), fractional urinary fluoride excretion (%) and fractional fluoride retention (%) among 4-year-olds (n=61) by area.

Table 2. Estimated Mean (SD) fluoride intake (mg/kg bw/d), urinary fluoride excretion (mg/kg bw/d), fluoride retention (mg/kg bw/d), fractional urinary fluoride excretion (%) and fractional fluoride retention (%) among 8-year-olds (n=63) by area.

Table 3. Mean and 95% confidence interval of difference in fluoride intake (mg/kg bw/d), urinary fluoride excretion (mg/kg bw/d), fluoride retention (mg/kg bw/d), fractional urinary fluoride excretion (%) and fractional fluoride retention (%) between 4- (n=61) and 8-year-olds (n=63).

Figure 1: Relationship between total daily F intake (TDFI: mg/kg bw/d) and daily urinary F excretion (DUFE: mg/kg bw/d) for 4-year-olds (n=61) and 8-year olds (n=63).

Figure 2: Relationship between total daily F intake (TDFI: mg/kg bw/d) and daily F retention (DFR: mg/kg bw/d) for 4-year-olds (n=61) and 8-year olds (n=63).

Figure 3: Relationship between total daily F intake (TDFI: mg/kg bw/d) and fractional F retention (FFR: %) for 4-year-olds (n=61) and 8-year olds (n=63).

Figure 4. Fluoride retention (%) by age, based on data from the present study and relevant literature. [24, 34-37]

Table 1. Estimated Mean (SD) fluoride intake (mg/kg bw/d), urinary fluoride excretion (mg/kg bw/d), fluoride retention (mg/kg bw/d), fractional urinary fluoride excretion (%) and fractional fluoride retention (%) among 4-year-olds (n=61) by area.

	Area			
	Higher F area		Lower F area	
	Urban[1] (n=16)	Rural[2] (n=15)	Urban[3] (n=17)	Rural[4] (n=13)
Total daily F intake (mg/kg bw/d)	0.059 (0.029)[a]	0.385 (0.184)[b]	0.050 (0.019)[a]	0.062 (0.022)[a]
Total daily F intake from diet (mg/kg bw/d)	0.041 (0.026)[a]	0.362 (0.181)[b]	0.033 (0.012)[a]	0.033 (0.012)[a]
Drinks	*0.020 (0.025)[a]*	*0.094 (0.097)[b]*	*0.003 (0.004)[a]*	*0.010 (0.011)[a]*
Food	*0.021 (0.007)[a]*	*0.268 (0.158)[b]*	*0.028 (0.012)[a]*	*0.023 (0.008)[a]*
Contribution of diet to total daily F intake (%)	67 (17)[a]	93 (6)[b]	68 (15)[a]	54 (16)[a]
Total daily F intake from toothpaste ingestion (mg/kg bw/d)	0.018 (0.011)[*]	0.022 (0.014)[*]	0.016 (0.011)[*]	0.029 (0.016)[*]
Daily urinary F excretion (mg/kg bw/d)	0.031 (0.028)[a]	0.053 (0.030)[a,b]	0.025 (0.025)[a,c]	0.021 (0.010)[a,c]
Daily F Retention (mg/kg bw/d)	0.022 (0.037)[a]	0.293 (0.178)[b]	0.020 (0.028)[a]	0.035 (0.021)[a]
Fractional Urinary F excretion (%)	62 (52)[a]	18 (13)[b]	54 (58)[a,b]	37 (20)[a,b]
Fractional F Retention (%)	28 (52)[a]	72 (13)[b]	36 (58)[a,b]	53 (20)[a,b]

Variables with different letters are statistically significantly different at p<0.05 (post-hoc test). *ANOVA – No significant difference. (See Supplementary material available).

[1] Community ground water (ppmF) = 0.8-1.0; Range (median) drinking and cooking water (ppmF), respectively: ≤0.43 (0.01) ppmF, ≤0.40 (0.03) ppmF
[2] Community ground water (ppmF) = 2.0-3.0; Range (median) drinking and cooking water (ppmF), respectively: ≤3.67 (0.3) ppmF, ≤4.00 (0.33) ppmF
[3] Community ground water (ppmF) = 0.04-0.07; Range (median) drinking and cooking water (ppmF), respectively: ≤0.08 (<0.01) ppmF, ≤2.00 (<0.01) ppmF
[4] Community ground water (ppmF) = 0.06-0.07; Range (median) drinking and cooking water (ppmF), respectively: ≤0.60 (0.07) ppmF, ≤0.40 (0.01) ppmF

Table 2. Estimated Mean (SD) fluoride intake (mg/kg bw/d), urinary fluoride excretion (mg/kg bw/d), fluoride retention (mg/kg bw/d), fractional urinary fluoride excretion (%) and fractional fluoride retention (%) among 8-year-olds (n=63) by area.

	Area			
	Higher F area		Lower F area	
	Urban[1] (n=13)	Rural[2] (n=13)	Urban[3] (n=17)	Rural[4] (n=20)
Total daily F intake (mg/kg bw/d)	0.057 (0.045) [a]	0.326 (0.128) [b]	0.048 (0.038) [a]	0.043 (0.016) [a]
Total daily F intake from diet (mg/kg bw/d)	0.041 (0.039) [a]	0.307 (0.120) [b]	0.033 (0.012) [a]	0.030 (0.014) [a]
Drinks	*0.016 (0.024)* [a]	*0.073 (0.081)* [b]	*0.003 (0.004)* [a]	*0.013 (0.013)* [a]
Food	*0.025 (0.019)* [a]	*0.234 (0.096)* [b]	*0.030 (0.039)* [a]	*0.018 (0.010)* [a]
Contribution of diet to total daily F intake (%)	67 (16) [a]	94 (6) [b]	72 (16) [a]	68 (15) [a]
Total daily F intake from toothpaste ingestion (mg/kg bw/d)	0.015 (0.010)*	0.019 (0.016)*	0.012 (0.007)*	0.013 (0.006)*
Daily urinary F excretion (mg/kg bw/d)	0.017 (0.014) [a]	0.044 (0.018) [b]	0.022 (0.013) [a]	0.012 (0.007) [a]
Daily F Retention (mg/kg bw/d)	0.034 (0.041) [a]	0.249 (0.112) [b]	0.021 (0.034) [a]	0.027 (0.015) [a]
Fractional Urinary F excretion (%)	39 (33) [a]	17 (14) [a]	56 (34) [ab]	29 (19) [ac]
Fractional F Retention (%)	51 (33) [a]	73 (14) [a]	34 (34) [ab]	61 (19) [ac]

Variables with different letters were statistically significantly different at $p<0.05$ (post-hoc test). *ANOVA – No significant difference. (See Supplementary material available).

[1] Community ground water (ppmF) = 0.8-1.0; Range (median) drinking and cooking water (ppmF), respectively: ≤0.2 (0.01) ppmF, ≤0.20 (0.01) ppmF
[2] Community ground water (ppmF) = 2.0-3.0; Range (median) drinking and cooking water (ppmF), respectively: ≤3.0 (0.3) ppmF, ≤3.00 (0.47) ppmF
[3] Community ground water (ppmF) = 0.04-0.07; Range (median) drinking and cooking water (ppmF), respectively: ≤0.2 (0.01) ppmF, ≤1.00 (0.05) ppmF
[4] Community ground water (ppmF) = 0.06-0.07; Range (median) drinking and cooking water (ppmF), respectively: ≤0.50 (0.08) ppmF, ≤0.50 (0.07) ppmF

Table 3. Mean and 95% confidence interval of difference in fluoride intake (mg/kg bw/d), urinary fluoride excretion (mg/kgbw/d), fluoride retention (mg/kg bw/d), fractional urinary fluoride excretion (%) and fractional fluoride retention (%) between 4- (n=61) and 8-year-olds (n=63).

|	Mean (SD)		Mean (95% Confidence interval) of difference	P value
	4-year-olds (n=61)	8-year-olds (n=63)		
Total daily F intake (mg/kg bw/d)	0.137 (0.169)	0.106 (0.130)	+0.031 (-0.022, +0.085)	0.247
Total daily F intake from diet (mg/kg bw/d)	0.116 (0.167)	0.091 (0.126)	+0.025 (-0.028, +0.078)	0.352
Drinks	0.031 (0.061)	0.024 (0.046)	+0.007 (-0.012, +0.026)	0.457
Food	0.085 (0.131)	0.67 (0.098)	+0.017 (-0.024, +0.059)	0.398
Contribution of diet to total daily F intake (%)	71 (19)	74 (17)	-3 (-10, +3)	0.330
Total daily F intake from toothpaste ingestion (mg/kg bw/d)	0.021 (0.013)	0.014 (0.010)	+0.007 (+0.002, +0.011)	0.002
Daily urinary F excretion (mg/kg bw/d)	0.032 (0.027)	0.022 (0.017)	+0.010 (+0.002, +0.018)	0.013
Daily F Retention (mg/kg bw/d)	0.091 (0.147)	0.073 (0.107)	+0.018 (-0.027, +0.064)	0.433
Fractional Urinary F excretion (%)	44 (44)	36 (30)	+8 (-5, +21)	0.247
Fractional F Retention (%)	46 (44)	54 (30)	-8 (-21, +5)	0.238
Urine volume (ml/kg bw/d)	24.8 (10.0)	27.9 (14.8)	-3.1 (-7.6, +1.4)	0.178

Figure 1: Relationship between total daily F intake (TDFI: mg/kg bw/d) and daily urinary F excretion (DUFE: mg/kg bw/d) for 4-year-olds (n=61) and 8-year olds (n=63).

4-year-olds: DUFE (mg/kg bw/d)=[0.046 x TDFI(mg/kg bw/d)]+0.026; (r) = +0.29, P=0.025.

8-year-olds: DUFE (mg/kg bw/d)=[0.085 x TDFI(mg/kg bw/d)]+0.013; (r) = +0.64, P <0.001.
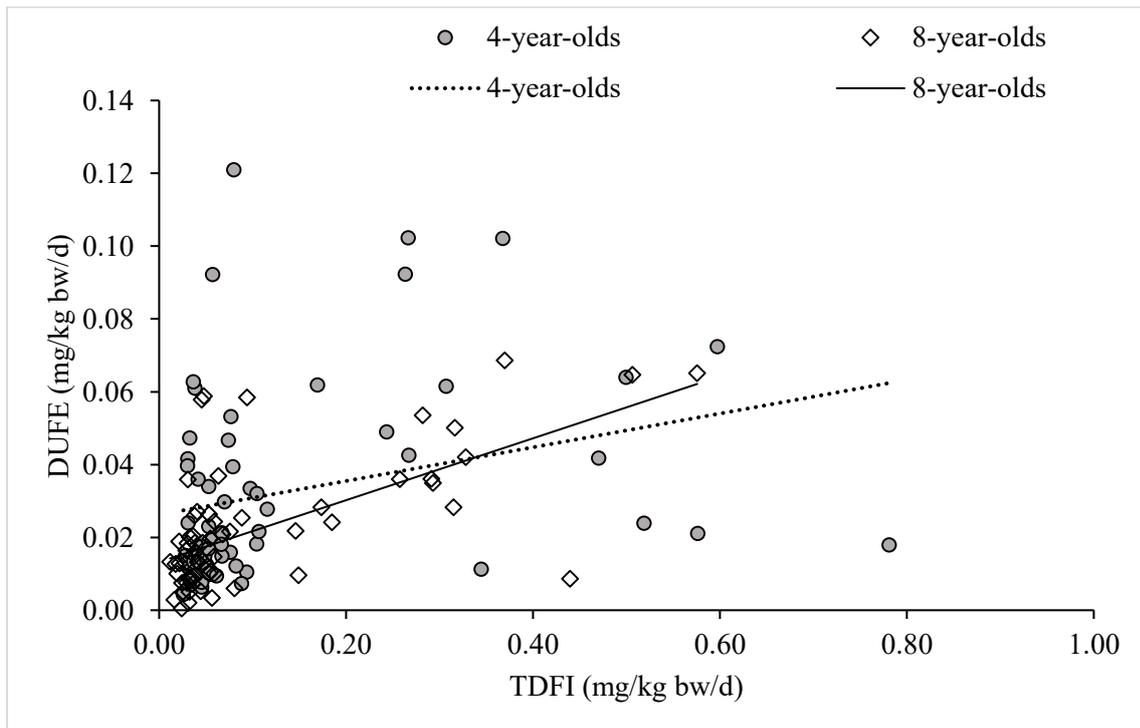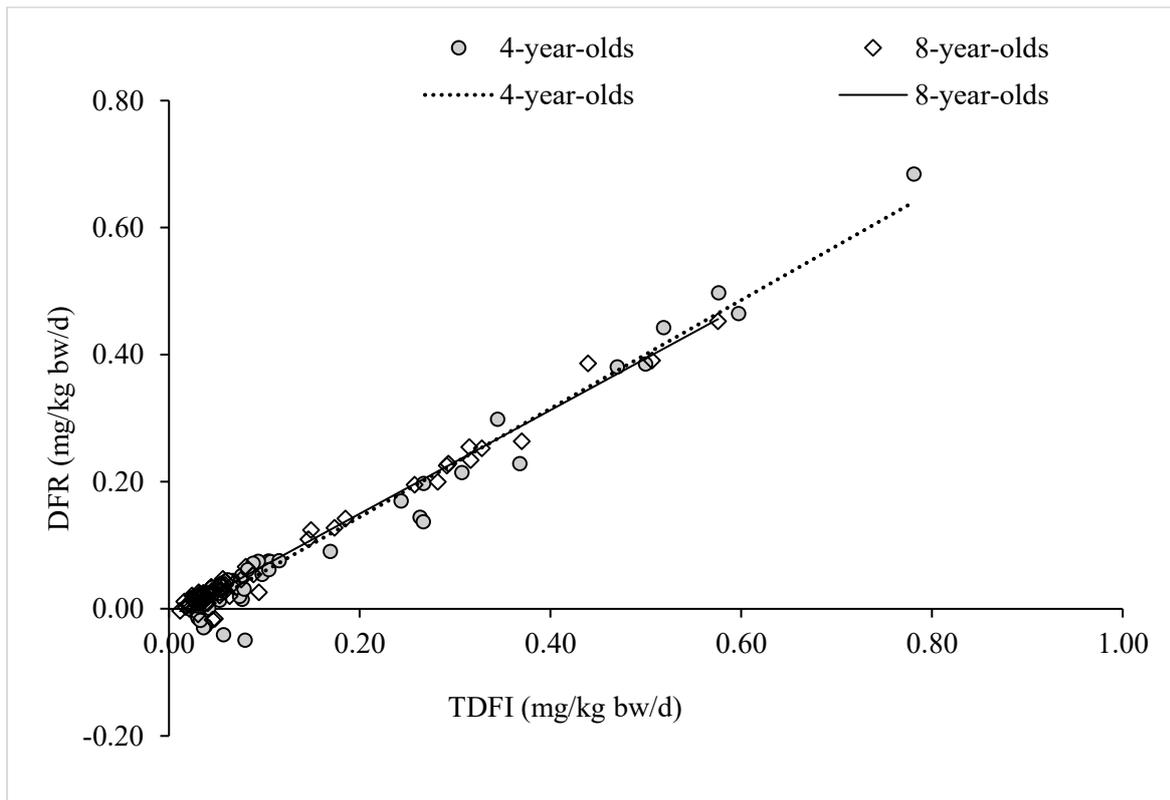
Figure 2: Relationship between total daily F intake (TDFI: mg/kg bw/d) and daily F retention (DFR: mg/kg bw/d) for 4-year-olds (n=61) and 8-year olds (n=63).

4-year-olds: DFR (mg/kg bw/d)=[0.854 x TDFI(mg/kg bw/d)] -0.026; (r) = +0.98, P <0.001.

8-year-olds: DFR (mg/kg bw/d)=[0.815 x TDFI(mg/kg bw/d)] -0.013; (r) = +0.99, P <0.001.

Figure 3: Relationship between total daily F intake (TDFI: mg/kg bw/d) and fractional F retention (FFR: %) for 4-year-olds (n=61) and 8-year olds (n=63).
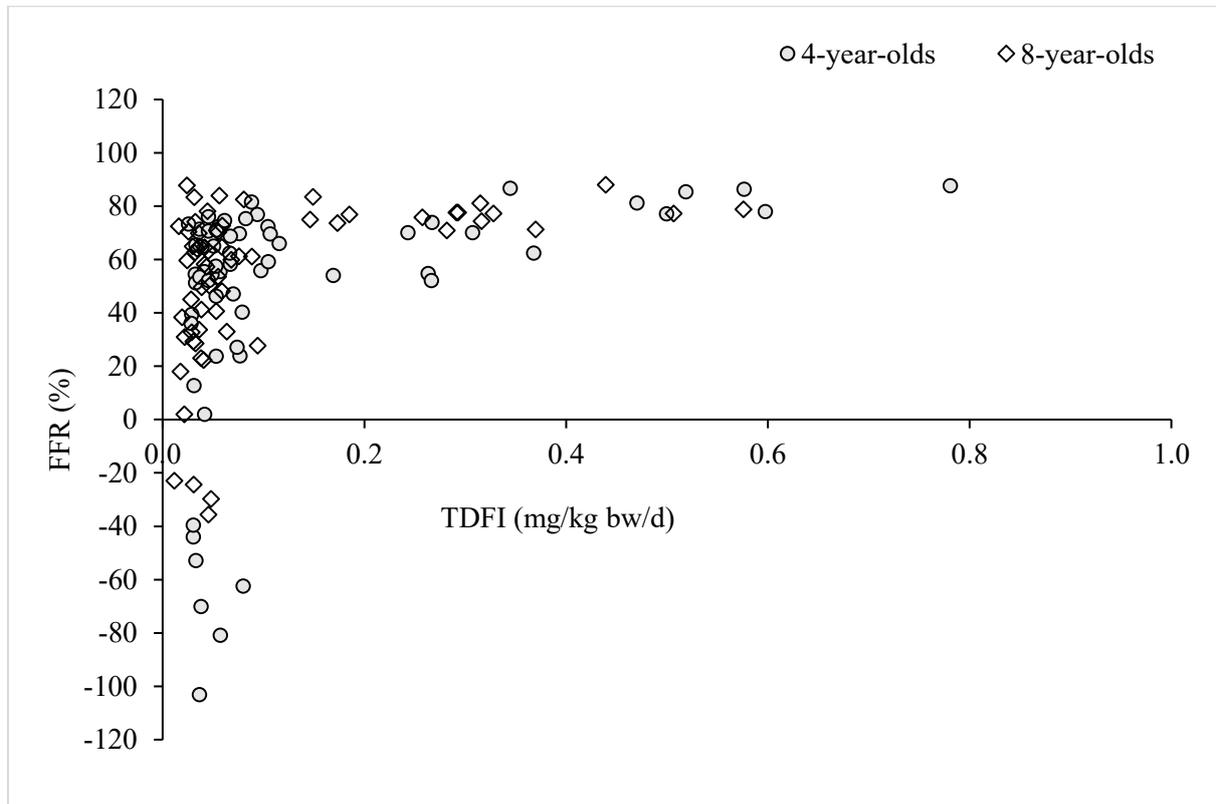
Figure 4. Fluoride retention (%) by age, inferred from data from the present study and relevant literature. [23, 33-36]