Biomarkers for the assessment of exposure to fluoride in adults

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ABSTRACT

To monitor deficient or excessive intakes of biologically available fluoride (F), various biological samples have been tested for use as biomarkers of human exposure to F. Most such studies have concerned children and often have only involved measurement of F in one or two types of sample. The present study investigated the relationships of F concentrations in biomarkers of F exposure including plasma, saliva, hair, finger- and toenails, and daily urinary fluoride excretion (UFE) with the total daily fluoride intake (TDFI) of adults. TDFI was assessed in 60 healthy adults, aged >= 20 years; 31 lived in a low-fluoride water area (LFA, 0.04 mg F/L) and 29 in a high-fluoride water area (HFA, 3.05 mg F/L) of Nigeria. All volunteers provided at least one biomarker sample from the above list and completed a questionnaire to evaluate fluoride intake from the diet and toothpaste ingestion. TDFI, UFE and F concentrations of biomarkers were statistically significantly higher in the HFA than in the LFA. There were strong statistically significant positive correlations between TDFI and UFE ($\rho = 0.730$, $p < 0.001$); plasma F ($\rho = 0.729$, $p < 0.001$); fasting whole saliva F ($\rho = 0.653$, $p < 0.001$) and hair F ($\rho = 0.603$, $p < 0.001$). The statistically significant positive correlations between TDFI and fingernail F ($\rho = 0.502$, $p < 0.001$) and between TDFI and toenail F ($\rho = 0.556$, $p < 0.001$) were moderate. In conclusion, this study has indicated the usefulness of 24h UFE as well as fluoride concentration in plasma, fasting whole saliva and hair as biomarkers of contemporary or sub-chronic F exposure in groups of adults. However, they do not appear to have the necessary sensitivity to predict F exposure in individuals.

INTRODUCTION

Fluoride (F) is a long-established treatment for the control and prevention of dental caries lesions. However, an increase in the prevalence of dental fluorosis in children, ascribed to excessive ingestion of F during tooth development, has received increasing attention in recent years [Khan et al., 2005, Harding and O'Mullane, 2013]. In contrast, skeletal fluorosis, a serious condition that affects adults resulting from chronic ingestion of large amounts of F over many years during periods of bone modelling and/or remodelling [Zohoori and Duckworth, 2017], has been less well studied [Everett, 2011]. The total quantity of ingested F is the most important factor, determining the clinical course of fluorosis. In adults, the most important sources of F exposure are diet (including water) and airborne F in urbanised areas from industrial sources such as production and use of phosphate fertilisers and phosphate ores, as well as aluminium, steel and other metal production facilities [Zohoori and Duckworth, 2017]. Dentifrice can be another potential source of F intake in adults, depending on dentifrice F concentration and quantity as well as rinsing regimen and brushing duration [Creeth et al., 2013]. The recommendation of "not rinsing" after brushing [Pitts et al., 2012], which has been endorsed by many organisations such as the 'Public Health England' [PHE, 2017], therefore increases the amount of F retained in the mouth and consequently ingested.

Given the difficulties in measuring an individual's exposure to F from all sources, the World Health Organization [World Health Organization, 1994] proposed the use of F biomarkers as an alternative approach to monitor intake of bioavailable F. Biological samples such as teeth, bone, nails, hair, blood and saliva have been useful in estimating exposure to F [Whitford, 1996]. Fluoride concentrations in bodily fluids (e.g., urine, plasma, serum, saliva) are probably most suitable contemporary markers for evaluating recent F exposures. In contrast, hair and nails (fingernails and toenails) may be useful for measuring exposure over longer periods of time. Extensive human studies have been conducted on biomarkers of contemporary and recent F exposure and a summary of the research has been published [Pessan and Buzalaf, 2011, Rugg-Gunn et al., 2011]. To date, no firm conclusions can be made on which biomarkers are the most suitable indicators of F utilisation and accumulation in the body.

Recently, we published a comprehensive study that investigated the value of various F biomarkers in two groups of children living in areas of Nigeria with low- and high concentrations of F in the drinking water, respectively [Idowu et al., 2020]. Daily urinary F excretion (UFE) and F concentrations in plasma, whole saliva, hair and nails (fingernails and toenails) were measured and related to total daily F intake (TDFI) values evaluated by questionnaire. UFE, plasma fluoride and toenail fluoride were strongly positively correlated with TDFI, whereas corresponding correlations for fingernails, saliva and hair were less significant.

The present work involved adults, for which less published data is available than for children. The work reported is related to the previous study on children [Idowu et al., 2020]. Data for adults were not included in that report, mainly to be able to discuss the findings for each age group more clearly, which would not have been possible in a combined condensed report. There is no robust evidence on the usefulness of the various F biomarkers yet in adults. No reports have compared F amounts in urine, whole saliva, plasma, hair and nails (fingernail and toenail) among adults with customary F intake and exposed to a wide range of F sources. North Central Nigeria, where the natural F concentration in groundwater varies considerably, from <1 to 4 μgF/ml, [Uriah et al., 2014], provided a suitable study location.

MATERIALS AND METHODS

Parents of children enrolled in the previous study [Idowu et al., 2020] were invited to participate in this cross-sectional observational study. All were healthy adults aged 20-35 years, who had lived in either a low-F water area (LFA; Bokkos Local Government Area (LGA)) or a high-F water area (HFA; Langtang North LGA) since birth. Written informed consent was obtained from all participants who volunteered to take part.

The mean (SD) F concentration of the drinking water for the LFA and the HFA was 0.04 (0.02) and 3.05 (1.10) mg/L, respectively [Idowu et al., 2020].

Ethical approval

The study was approved by the School of Health and Social Care Ethics Committee, Teesside University (Study number 065/15) and the Jos University Teaching Hospital Ethics Committee, JUTH,

Plateau State, Nigeria (JUTH/DCS/ADM/127/XIX/6408). Approval was also obtained from health establishments and local government authorities as well as the district heads and relevant community heads of the selected villages prior to the commencement of the study.

Data and Sample Collection

Dietary information and the various F biomarker samples (urine, blood, saliva, hair, fingernails and toenails) were collected during two home visits, details of which are given in our previous paper [Idowu et al., 2020]. In summary, to estimate TDFI (i) a validated oral hygiene habit questionnaire was used to estimate F intake from toothpaste ingestion; and (ii) a validated food frequency questionnaire (FFQ) was also administered to estimate F intake from diet. None of the participants lived in areas of F pollution. Samples of toothpaste brands used as well as the most frequently consumed food and drinks identified from a food questionnaire were obtained either directly from participants' homes or bought from supermarkets and analysed for F content. Hair samples were collected by cutting hair strands as close to the scalp as possible from the back of the head. Samples of unstimulated whole saliva were collected by asking the participants to drool into plastic vials; and a 5-mL blood sample was collected by a qualified nurse. Participants were also instructed to (i) clip their nails from all digits after cleaning the nails; and (ii) collect their urine samples over a period of 24 hours.

Sample Preparation and Analysis

Details of sample preparation are given elsewhere [Idowu et al., 2020]. Water and urine samples were analysed directly by F ion-selective electrode, whilst food, toothpaste, plasma, whole saliva, hair and nail samples were analysed after overnight hexamethyldisiloxane-acid diffusion [Martínez-Mier et al., 2011].

Data Preparation

The completeness of 24h urine samples was checked by comparing urinary flow rate (mL/h) with the World Health Organization [World Health Organization, 2014] reference ranges for ≥6-year-olds (9-300 mL/h). Participants with a urine flow rate outside this range were excluded from data analysis. Daily urinary F excretion (DUFE; mg/d) was estimated by multiplying the 24h urine volume by the F concentration of the urine sample.

Each participant's daily dietary F intake was estimated by multiplying the F concentration (mg/kg) of each food and drink by the corresponding amount (kg) consumed per day and then summing these values to derive a total in mg/d and on a body weight basis (mg/kg bw/d). F ingestion from toothpaste (mg/d) was estimated by multiplying the pictorially [Levy and Zarei-M, 1991] estimated weight of toothpaste used per brushing (mg) by its F concentration and frequency of use. The obtained value was multiplied by 26%: the mean % of fluoride retained per toothbrushing session reported in a recent comprehensive toothbrushing study in adults [Creeth et al., 2013]. Total daily F intake (TDFI; mg/day) was estimated from diet and ingestion from toothpaste by summing up F intake from these two sources. TDFI and DUFE were also calculated based on body weight (mg/kgbw/d) by dividing these values (mg/d) by body weight (kg).

Statistical Analysis

Sample size: A power analysis was undertaken to estimate appropriate sample size based on previous studies [Schamschula et al., 1985, Levy et al., 2004], which showed a highly significant difference in the level of F in the biomarkers with increasing water F concentration. It was estimated that 20 participants per area would be needed for 90% power and an assumed statistical significance level α of 0.05. However, 62 participants were invited to allow for drop outs.

The data were analysed descriptively using SPSS version 22. Independent t-tests were used to compare means for each parameter between the LFA and HFA groups. Pearson's correlation coefficient was used to examine the relationships between the various biomarkers and TDFI. The strength of the correlation (ρ) was then evaluated using the following categories [Evan, 1996]: very weak (0.00-0.19), weak (0.20-0.39), moderate (0.40-0.59), strong (0.60-0.79) and very strong (0.80-1.00).

RESULTS

In total, 60 participants provided at least one sample; 31 in the LFA and 29 in the HFA. There was no statistically significant difference in the age and weight of the participants living in the LFA (33.1 years and 67.5 kg respectively) and in the HFA (34.6 years and 64.8 kg respectively).

Of the 60 participants who took part in the study: 55 provided complete 24h urine, 54 whole saliva, 60 plasma, 56 hair, 54 fingernail and 50 toenail samples. Table 1 presents the mean (SD) of TDFI, 24h

UFE and F concentrations of the biomarkers. Comparison between the two F water areas indicated no difference in F intake from toothpaste ingestion, whereas F intake from diet and TDFI was higher ($p < 0.001$) in the HFA compared with the LFA. In addition, 24h UFE and F concentrations of biomarkers were statistically significantly higher in the HFA than in the LFA.

The linear relationships between the various biomarkers and TDFI are presented in Figures 1 to 4. There were strong statistically significant positive correlations between TDFI and i) 24h UFE (Figure 1: ρ=0.730, $p<0.001$); ii) plasma F (Figure 2: ρ=0.729, $p<0.001$); fasting whole saliva F (Figure 2: ρ=0.653, $p<0.001$) and iv) hair F (Figure 4: ρ=0.603, $p<0.001$). The statistically significant positive correlations between TDFI and fingernail F (Figure 3: ρ=0.502, $p<0.001$) and between TDFI and toenail F (Figure 3: ρ=0.556, $p<0.001$) were moderate.

For UFE, and F in plasma, saliva, hair, fingernails and toenails, the main effect of fluoride area yielded F ratios of 119, 185.9, 34.2, 61.5, 87.2, 64.2, respectively, indicating significant differences ($p < 0.001$) between the HFA and the LFA.

DISCUSSION

Monitoring F exposure and examining the population for signs of excessive exposure have been recommended by many including the World Health Organization [World Health Organization, 2014]. The risk of dental and skeletal fluorosis might be determined more easily if there is a valid predictor of F exposure from measurement of its biomarkers. The current study reports the association between total exposure to F and several biomarkers, including 24h urinary F excretion as well as F concentration in plasma, saliva, hair and nails, in adults exposed to a wide range of F, from low water to endemic F areas. To our knowledge, this is the first report on such associations in adults residing in endemic F areas.

The number of participants in our study is lower than in reports from Mexico (239 adults aged 18-77 years [Jiménez-Córdova et al., 2018] and 872 pregnant women [Thomas et al., 2016]) and Ethiopia (386 participants aged 10-50 years) [Rango et al., 2017]. However, those studies measured a maximum of two biomarkers ('urine', 'urine and fingernails', and 'urine and plasma', respectively), whereas we collected six different types of biomarkers from each participant (i.e. 329 biomarker samples, in total). Dietary F intake has been mainly assessed using three methods: duplicate plate collection [Rojas-Sanches et al., 1999; Paiva et al., 2003], food diary [Zohoori et al, 2019] and questionnaire [Ibiyemi et al., 2018]. However, comparisons between methods have suggested that all three are suitable for estimating dietary F intake at a group level [Martinez-Mier et al., 2004; Omid et al., 2015].

As expected, the dietary F intake was significantly higher in the HFA compared to the LFA. The average proportion of F from diet in our study was 88% in LFA and 97% in HFA (Table 1) in comparison with the corresponding figure of 91% reported for ≥19 y old Chileans with a drinking water F concentration of 0.6 mg/L [Villa et al., 2004]. TDFI in the LFA (0.033 mg/kgbw/d) was similar to the figure of 0.037 mg/kgbw/d reported among adults, aged 19-30 y, in Chile [Villa et al., 2004].

Our results show that the F concentration of water (i.e. F area) was the most important factor influencing the 24 UFE and F concentration in the other investigated biological markers (saliva, plasma, finger- and toe-nails). A study in five Brazilian communities, with drinking water F concentrations of 0.09 to 1.68 mg/L also reported geographical area and water F concentration as the strongest influencers in whole

and ductal saliva F concentrations, respectively [Fukushima et al., 2011] as well as nail F concentrations [Fukushima et al., 2009].

When we examined the relationship between TDFI and various biomarkers, we found 24h urine (Fig 1) and plasma (Fig 2) as the strongest predictors of F exposure in adults. Our findings are in agreement with those by Villa et al [Villa et al., 2010], who reported a highly significant linear relationship between TDFI and 24h UFE, with results obtained from pooled data of six independent studies in adults (19-75y). Plotting mean values of plasma F concentration against F dose, from eleven studies, [Rugg-Gunn et al., 2011] also showed a linear relationship between TDFI and plasma F concentration, although they did not examine the statistical significance.

Among different biomarkers of F exposure, fasting plasma has been acknowledged as the gold standard. However, 24h UFE can be considered as a more appropriate contemporary biomarker of F exposure due to being a non-invasive method and the fact that varying proportions of a given F dose are completely excreted with the urine in <24h. Due to logistical challenges of collecting 24h urine samples, spot urine collection, normalised for creatinine excretion using creatinine reference values, has been proposed as a simpler alternative to 24-hour urine sample collection [Zohoori and Maguire, 2018]. In the present study, no spot urine sample was collected, as a further potential F biomarker, due to the lack of resources including reference values for creatinine concentration in urine in our study population. Although the value of urinary fluoride to creatinine ratio in a spot urine sample as a useful systemic F exposure monitoring tool has been shown in children, no study has investigated its validity in adults. Therefore, future studies are needed to examine this alternative approach in adults living in different geographical locations with different dietary habits.

F concentration in fasting plasma is a function of several physiological variables, including the F concentration in the exchangeable pool of bone, the rates of bone accretion and resorption, and the renal clearance of F. Generally, levels of F in urine are proportional to plasma concentrations, but they fluctuate more due to the effects of urinary flow rate and the extent of tubular F reabsorption. UFE tends to increase with urinary pH, and therefore environments or conditions that chronically affect urinary pH, including diet, drugs, altitude and certain diseases, e.g. chronic obstructive pulmonary disease, might affect the rate of urinary excretion of F [Buzalaf, 2011].

Our results showed a fasting plasma F concentration of 0.031 µg/mL in the LFA and 0.121 µg/mL in the HFA. For low water F concentrations (≤ 0.25 mg/L), fasting plasma F concentrations in adults (20-38y) have ranged from 0.010 to 0.020 µg/mL; whereas in areas with water F concentrations of 2.00-2.73 mg/L they have ranged from 0.100 to 0.137 µg/mL [Rugg-Gunn et al., 2011]. Independent of F dose, there are other factors which could impact the concentration of F in plasma, including site of blood collection, age, acid- base balance, altitude, haematocrit and genetic background [Buzalaf, 2011].

Our study found a relatively strong correlation (ρ =0.653) between F in fasting whole saliva and TDFI. Some studies have also shown whole saliva might be a good indicator of recent topical F exposure [Zero et al., 1988, Duckworth et al., 1991, Cury et al., 2005, Duckworth et al., 2009, Székely et al., 2010]. However as a F biomarker, submandibular/sublingual duct saliva has been regarded as a superior F biomarker than parotid duct saliva, and both more preferable than whole saliva [Rugg-Gunn et al., 2011]. On the other hand, collection of ductal saliva is technically more difficult and therefore it becomes inevitable to investigate the potential of whole saliva as a biomarker of F exposure. In a series of experiments, the ratio for F concentrations in whole saliva and plasma was reported to be 1.10 [Oliveby et al., 1989], implying that whole saliva had acquired F from the oral environment. However, our study found a lower F concentration in fasting whole saliva than in fasting plasma. Studies have shown that diets and fluoridated dentifrice, amount and frequency of fluid consumption, as well as other factors including soft tissue retention of F, dissolution of CaF, crevicular fluid and oral bacteria [Boros et al., 2001, Martinez-Mier and Soto-Rojas, 2010], could have a great influence on the level of F in the oral cavity, which would be reflected in the concentration of F in whole saliva.

Hair and nails, as biomarkers of F exposure, have a huge advantage due to their ease of collection, storage and non-invasiveness. Since F concentrations in hair and nail-clippings are directly related to the average F intake and plasma F concentration during the period when they were formed and not to the exposure that occurred during the day of sampling, they could provide information on sub/chronic exposure to F.

Our study is the first to report the relationship between total F exposure and F concentration in hair. The relatively strong correlation between these parameters, observed in our study, suggests that hair could be a useful biomarker for evaluating exposure to F in adults. Strong correlations between hair F

concentration and F in water have also been reported for children [Mandinicet al., 2010, Antonijevicet al., 2016]; whereas the corresponding correlation for adults was reported as weak among Poles [Czarnowski et al., 1999]. However, the mean hair F concentration of adults living in the HFA in our study (5.69 μg/g) was similar to the value (5.4 μg/g) found among those living in areas polluted with F compounds in the air (> 0.5 mg HF/m^3 due to a phosphate fertiliser plant which used fluoroapatites in manufacturing) [Czarnowski and Krechniak, 1990].

A moderate correlation was found between F concentration in finger- and toe-nails and TDFI in our study. Given that toenails grow slower than fingernails [Whitford et al., 1999], they might represent different time frames of exposure to F. Fingernails have been suggested as an unreliable biomarker of F exposure from dentifrice in children due to the lack of sensitivity to detect differences in the exposure dose of 0.04 mgF/kg bw/d [Lima-Arasti et al., 2010]. On the other hand, toenails have been proposed as a more reliable F biomarker than fingernails due to being less susceptible to external contamination [Pessan and Buzalaf, 2011]. Although water F concentration has been reported to exert the most influence on F concentration of finger- and toenails, older age groups (≥ 30y) and females have been found to have significantly higher nail F concentrations than younger age groups and males [Fukushima et al., 2009]. In contrast, a recent study [Sah et al., 2020] reported a statistically significantly higher F concentration in the fingernails of parents than of their children, whereas no statistically significantly difference in toenail F concentration between parents and children was observed.

Comparison of our findings for adults, as reported here, with their children (as reported previously [Idowu et al., 2020]), found no effect of age group for F in plasma, saliva and toenails; whereas there were significant differences between children and adults for UFE, fingernail and hair F concentrations. Age is a factor that influences UFE and consequently retention of F in the body. Approximately 65% of the F absorbed each day by healthy adults is excreted in urine whereas the corresponding value is 45% in children [Villa et al., 2010]. On the other hand, the proportion of F retained in calcified tissues in children is higher than the corresponding values for adults. Increased retention by the developing skeleton in children appears to be due almost entirely to the rich blood supply and comparatively large surface area of bone crystallites, which are smaller, more loosely organized, and more numerous than those of mature bone [Whitford, 1994]. Peak plasma F concentration and the areas under the time-

plasma F concentration curves (AUC) are also directly related to age during the period of skeletal development [Whitford, 1994].

Generally, our findings show a wide variation in F concentration for all the different biomarkers, which is in line with reported large variations in pharmacokinetic variables between individuals in previous studies [Maguire et al., 2005]. A study by Ekstrand [Ekstrand, 1978] of a family of five, aged 10 to 38 years old, with the same diet also showed a large variation in plasma F concentration and urinary F excretion between family members and a much greater within-individual variation during the day. Some of the relatively wide variation in pharmacokinetic variables between subjects might be related to factors such as variation in the amount of consumed water, type of diet (e.g. vegetarian vs meat-based diet), dietary and oral hygiene habits, and external contamination among participants; as well as interpersonal variation in the amount of absorbed, circulated, metabolised and retained F in the body.

In conclusion, our study highlights 24h urinary F excretion as well as F concentration in plasma as the most promising biomarker(s) of contemporary or sub-chronic F exposure. These biomarkers might help health authorities to improve health management/prevention strategies by better prediction of fluoride exposure at an early stage. However, it should be noted that while F concentrations in these body fluids/tissues could be considered as useful biomarkers of F exposure for a group of people, they do not appear to have the necessary sensitivity to predict F exposure for individuals.

The risk of dental and skeletal fluorosis might be determined more easily if there is a valid predictor of F exposure from measurement of its biomarkers.

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Statement of Ethics

The study was approved by the School of Health and Social Care Ethics Committee, Teesside University (Study number 065/15) and the Jos University Teaching Hospital Ethics Committee, JUTH, Plateau State, Nigeria (JUTH/DCS/ADM/127/XIX/6408). Approval was also obtained from health establishments and local government authorities as well as the district heads and relevant community heads of the selected villages prior to the commencement of the study. Written informed consent was obtained from each participant before taking part in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

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Authors' Contributions

FVZ, OSI conceived and designed the study; OSI collected and analysed the samples; FVZ supervised the project with help from RMD and RAV; FVZ and OSI analysed the data and RMD and RAV contributed to the interpretation of the results; FVZ and RMD took the lead in writing the manuscript. All authors read, provided critical feedback and approved the submitted paper.

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Figure Legends:

Fig 1. Relationship between total daily F intake (TDFI) and 24h urinary F excretion (UFE) (n=55).

24h UFE (mg/kgbw/d) = 0.007 + [0.887 x TDFI (mg/kgbw/d)]; ρ=0.730; p<0.001

Fig 2. Relationship between total daily F intake (TDFI) and i) plasma (n=60, "o, ……..") and ii) saliva (n= 54, "Δ, _____") F concentration.

Plasma F (µg/mL) = 0.035 + [0.560 x TDFI (mg/kgbw/d)]; ρ =0.729; p<0.001

Saliva F (µg/mL) = 0.018 + [0.555 x TDFI (mg/kgbw/d)]; ρ =0.653; p<0.001

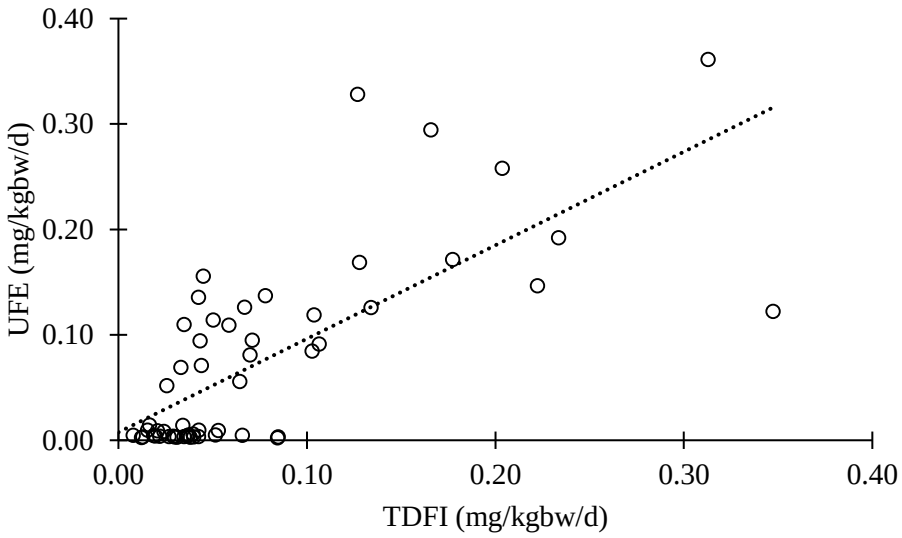
Fig 3. Relationship between total daily F intake (TDFI) and i) toe-nail (n=54, "o, ___"), and ii) finger-nail (n= 50, "x, - - -") F concentration.

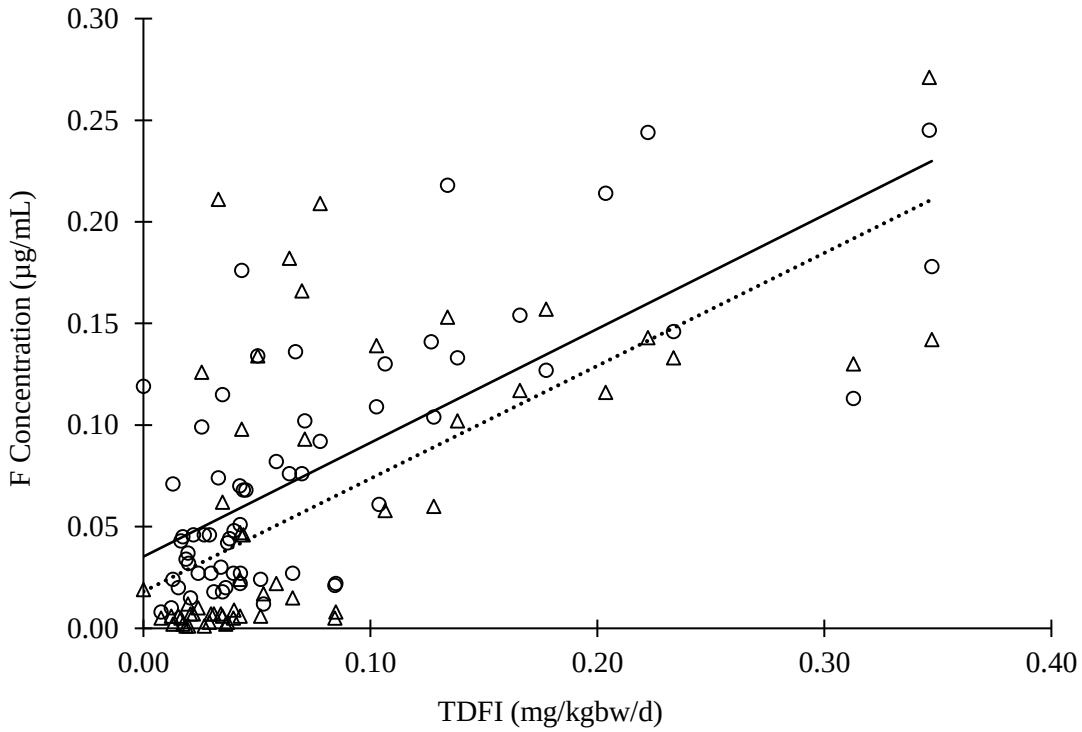
Fingernail F (µg/g) = 3.80 + [30.04 x TDFI (mg/kgbw/d)]; ρ =0.502; p<0.001

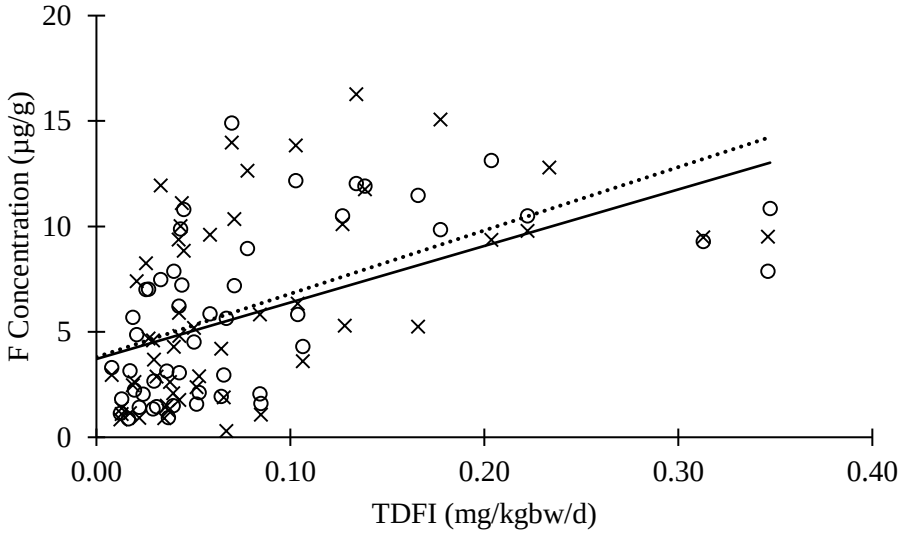
Toenail F (µg/g) = 3.71 + [26.81 x TDFI (mg/kgbw/d)]; ρ = 0.556; p<0.001

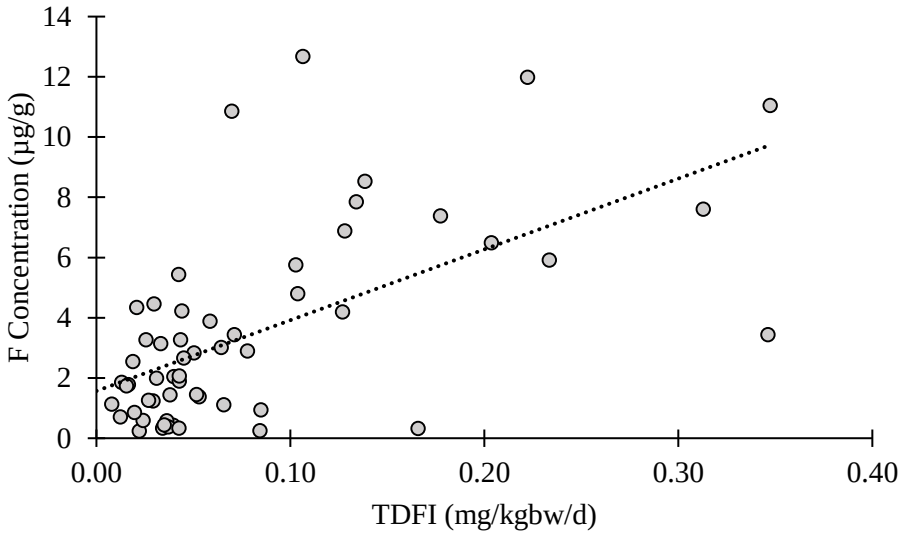
Fig 4. Relationship between total daily F intake (TDFI) and hair F concentration (n=56).

Hair F (µg/g) = 1.57 + [23.51 x TDFI (mg/kgbw/d)]; ρ =0.603; p<0.001


0.40
0.30
0.20
0.10
0.00
UFE (mg/kgbw/d)
0.00
0.10
0.20
0.30
0.40
TDFI (mg/kgbw/d)


0.30
0.25
0.20
0.15
0.10
0.05
0.00
F Concentration (µg/mL)
0.00
0.10
0.20
0.30
0.40
TDFI (mg/kgbw/d)


20
15
10
5
0
F Concentration (µg/g)
0.00
0.10
0.20
0.30
0.40
TDFI (mg/kgbw/d)


F Concentration (µg/g)
14
12
10
8
6
4
2
0
0.00
0.10
0.20
0.30
0.40
TDFI (mg/kgbw/d)