

1 **Pharmacokinetics of fluoride in human adults: the effect of exercise.**

2 Maria Mahmood ^a, Liane B Azevedo ^b, Anne Maguire ^c, M Buzalaf ^d, Fatemeh Vida Zohoori ^a

3 ^a School of Health and Life Sciences, Teesside University, Middlesbrough, UK

4 ^b School of Human and Health Sciences, University of Huddersfield, Huddersfield, UK

5 ^c School of Dental Sciences, Faculty of Medical Sciences, Newcastle University, Newcastle-upon-
6 Tyne, UK

7 ^d Bauru Dental School, University of Sao Paulo, Brazil

8

9

10

11 **Running title:** Effect of exercise on fluoride metabolism

12 **Keywords:** fluoride, renal excretion, exercise intensity

13 *** Corresponding author**

14 Professor FV Zohoori

15 School of Health and Life Sciences, Teesside University

16 Middlesbrough, TS1 3BA, UK

17 Tel: +44 (0) 1642 342973

18 Fax: +44 (0) 1642 342770

19 Email: v.zohoori@tees.ac.uk

20

21 **Abstract:**

22 The literature is sparse in terms of the effect of exercise on the pharmacokinetics of fluoride (F) in
23 humans. In a 4-treatment repeated measures cross-over study, we investigated F pharmacokinetics
24 following no exercise (control) and three exercise intensity conditions (light, moderate and vigorous)
25 in healthy adults. At a pre-experimental session, 8 participants (18-30y) residing in a non-fluoridated-
26 area, underwent a $VO_{2\max}$ test to guide the three exercise intensities for the experimental sessions.
27 Participants were on a F-free regime one week before and throughout the four experimental weeks.
28 We measured urinary F excretion (UFE), maximum plasma concentration (Cmax), lag time of Cmax
29 (Tmax), and Area Under the Curve (AUC) for plasma F concentration against time, following F
30 ingestion then no, light, moderate and vigorous exercise. Results showed no statistically significant
31 difference in Tmax among all sessions; whereas Cmax for moderate exercise (226.2ng/ml) was
32 significantly higher than for no (27.0ng/ml;p<0.001), light (105.6ng/ml;p=0.016) and vigorous
33 (94.2ng/ml; p=0.008) exercise. Mean AUC over 0 to 90 min following F ingestion was also
34 significantly higher in moderate exercise than for no (p<0.001), light (p=0.004) and vigorous
35 (p=0.001) exercise. Mean UFE over 0-14h was 638.8, 718.7, 574.6 and 450.5 μ g for no, light,
36 moderate and vigorous exercise, with no statistically significant differences among different sessions.
37 In conclusion, this human experimental study suggests that moderate exercise may increase the
38 fraction of F absorbed systemically which is therefore available to produce a biological effect. Future
39 studies should be conducted with larger samples, different age groups and using different F doses.

40

41

42 **1 Introduction**

43 While the effectiveness of topical exposure to fluoride (F) in the prevention of dental caries has been
44 well demonstrated, excessive exposure to systemic F can have some health side effects including
45 dental and skeletal fluorosis (ten Cate and Buzalaf, 2019). Undesirable health effects of F can be
46 related not only to the body's total F intake but, more importantly, to the extent of F retention in the
47 body. Genetic and environmental factors such as stage of skeletal development, acid-base balance and
48 exercise have been suggested to influence metabolism and body retention of F (Buzalaf and Whitford,
49 2011; Buzalaf, 2018). Understanding F metabolism and its physiological characterisation is therefore
50 very important if we are to avoid or minimise side effects of systemic F exposure.

51 The pharmacokinetics of F is mainly controlled by pH and storage in bone, because the coefficient of
52 permeability of lipid bilayer membranes to hydrogen fluoride (HF) is a million times higher than to F
53 ion (Buzalaf and Whitford, 2011). Therefore, factors affecting systemic pH (in cells, tissues and
54 fluids) could play an important role in the body's absorption, distribution, excretion and retention of
55 F. After absorption, F concentrations of plasma rise promptly due to the rapid absorption of F from
56 the stomach and reach their peak within 20-60 min. Plasma F concentration normally returns to pre-
57 ingestion levels during the next few hours depending on the F dose. Plasma F concentrations are not
58 homeostatically controlled and therefore fluctuate according to the F dose, body deposition and
59 excretion. Under normal conditions, almost 60% of a healthy adult's and 45% of a healthy child's
60 daily absorbed F is excreted in urine and most (about 99%) of the body-retained F is associated with
61 calcified tissues (Buzalaf and Whitford, 2011).

62 F has been reported as one of a few known agents that can stimulate osteoblast proliferation (Palmer
63 and Wolfe, 2005). However, different doses of F display a biphasic effect on osteoclast cell viability,
64 differentiation, formation and function: a low F dose stimulates them, whereas a high dose inhibits
65 them (Yu *et al.*, 2018). Furthermore, a decline in expression of osteocytes but a rise in expression of
66 osteoblasts has been linked to exercise (Schwab and Scalapino, 2011), in particular weight-bearing
67 exercise (Willems *et al.*, 2017). Therefore, the pharmacokinetics of F may be influenced by alterations
68 in physiological responses to acute and chronic exercise. Changes in body F retention could be

69 important in terms of the effect of F on tooth and bone development and the timing of F ingestion
70 when fluorides are used in dental caries prevention.

71 The literature is sparse and contradictory in terms of the effect of exercise on pharmacokinetics of F in
72 humans. The only human experimental study, comparing F concentration in plasma and urine between
73 exercised and non-exercised groups, reported higher plasma F concentrations with moderate and
74 vigorous intensity exercise as well as a reduction in urinary F excretion with moderate exercise
75 compared with a non-exercised control in young adults (Zohoori *et al.*, 2015). Conversely, two animal
76 studies have reported a significant reduction in plasma F concentration in rats exposed to a one-hour
77 treadmill running exercise (Whitford, 1996; Lombarte *et al.*, 2013). More recently, an animal study
78 (Amaral *et al.*, 2018) reported no effect of high intensity training exercise on plasma F in fluorosis-
79 susceptible mice.

80 The aim of this present study was to investigate the F pharmacokinetics following no exercise and
81 three exercise intensity conditions (light, moderate and vigorous) in healthy adults. The objectives
82 were to compare urinary F excretion (UFE) and plasma F concentration among no, light, moderate
83 and vigorous exercise intensities.

84

85 **2 Methods:**

86 2.1 Participants

87 This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all
88 procedures involving human participants were approved by the Research Governance and Ethics
89 Committee, School of Health and Social Care, Teesside University (Protocol number 066/15). Prior to
90 the experiment, all participants provided written informed consent.

91 The target sample size for this exploratory study was eight participants, based on the only human
92 study by Zohoori *et al* (n=9) (Zohoori *et al.*, 2015) as well as the animal studies by Whitford (n=8)
93 (Whitford *et al.*, 1988), Lombarte *et al* (n=10) (Lombarte *et al.*, 2013) and Amaral *et al* (n=8) (Amaral
94 *et al.*, 2018).

95 The study participants were healthy adult volunteers, from both genders; aged between 18 and 35
96 years; weighing over 50 kg; with no history of acid-base disturbance and not receiving a therapeutic
97 diet. Participants had to be considered at least moderately active according to the International
98 Physical Activity Questionnaire (i.e. 5 or more days of moderate-intensity activity of at least 30 min
99 per day) (Craig *et al.*, 2003) and “ready” to engage in the prescribed exercise according to the
100 Physical Activity Readiness Questionnaire (American College of Sports Medicine, 2007). All
101 participants were residing in a non-fluoridated area, with a water F concentration of <0.3 ppm.

102 2.2 Experimental Design

103 This experiment was designed as a four-treatment repeated measures cross-over study, comparing
104 observations within individuals.

105 *Pre-experimental Procedures*

106 Participants were invited to attend a pre-experimental session, when height and weight was measured
107 using a stadiometer (Leicester Height Measure, Child Growth Foundation, London, United Kingdom)
108 and a calibrated scale (Seca 220, Seca Weighing and Measuring Systems, Germany). Each individual
109 participant underwent a $VO_{2\max}$ test, performed following a standard cycle ergometer protocol (Evans
110 and White, 2009) to determine the exercise intensities (light, moderate and vigorous) for the
111 experimental sessions. Following a 5-minute warm up at 25 Watts, the test started at 25 Watts with
112 the intensity being increased by 25 Watts every 2 minutes until exhaustion. Cycle ergometer power
113 output (PowerTap Cycleops400, USA) and participant’s Rate of Perceived Exertion (RPE) using the
114 CR-10 RPE scale (Noble *et al.*, 1983) were recorded every minute. Heart rate was monitored
115 throughout the exercise sessions with a heart rate monitor (Polar RS400, Polar Electro Oy, Finland)
116 and recorded at every minute during exercise. The three exercise intensities (light, moderate and
117 vigorous) were determined by using the v-slope method (Beaver *et al.*, 1986) and defined for each
118 participant as: (i) light intensity: one load below Ventilatory Threshold (VT) 1; (ii) moderate
119 intensity: the load at VT and; (iii) vigorous intensity: one load above VT.

120 In order to minimise F exposure from all other sources during the study, participants were placed on a
121 F-free regime one week before, as well as during, the whole experimental period. They were provided
122 with a F-free toothpaste to use and instructed to avoid drinking tea, beer and tap-water (if leaving their
123 stated residential area) and eating seafood during the washout and experimental periods (i.e. for a total
124 of five consecutive weeks). Participants were asked to refrain from performing exercise, other than
125 habitual walking, for 48h prior to and during experimental sessions.

126 *Experimental sessions*

127 After the one-week washout period, each participant underwent four randomly allocated experimental
128 sessions; one no-exercise (control) session and three exercise sessions at different intensities (light,
129 moderate and vigorous) with approximately a week's gap between sessions (Figure 1).

130 The study was conducted in an exercise laboratory at the same time of day for all experimental
131 sessions to control for circadian rhythms.

132 Participants attended the laboratory at around 9:00 am on each experiment day, having fasted
133 overnight. A venous cannula was inserted into the antecubital fossa of each participant's arm, by an
134 experienced nurse, for intravenous blood samples collection (as explained in the next section). A
135 baseline venous blood sample (5ml) was collected from each participant before they were provided
136 with a low-F breakfast ($<10\mu\text{gF}$) which comprised a cereal bar, a banana and fruit juice. In order to
137 control the influence of F from diet during the experimental sessions, the same standardised low F
138 breakfast was consumed by all participants at approximately the same time in each of their four
139 sessions.

140 After breakfast, all participants (in both control and exercise sessions) were given a 1mgF tablet
141 (Endekay Fluotabs 2.2mg NaF, Manx Pharma Ltd, Warwick, UK) to ingest. Participants then either
142 rested (control session) or undertook the exercise (exercise sessions) at approximately 9:30am.

143 Participants from the exercise group were fitted with a heart rate monitor belt (Polar RS400, Polar
144 Electro Oy, Finland). Participants warmed up for 5 minutes at a self-selected speed before initiating
145 the designated exercise intensity on the cycle ergometer for 20 minutes.

146 2.3 Sample collection

147 *Urine samples*

148 Pooled urine samples were collected by spontaneous voiding over a 24h cycle during four time
149 periods: 1) A nocturnal sample collected from midnight before the experimental (control or exercise)
150 session up until about 09.00am (Baseline, pre-F tablet/pre-exercise); 2) A '09.00am to 12.00pm'
151 sample during the experimental session (0-3h post-F tablet ingestion); 3) A '12.00pm to 17.00pm'
152 sample during the experimental session (3-8h post-F tablet ingestion) and; 4) A '17.00pm through to
153 just before bed-time' sample (~23.00pm) on the experimental day (8-14h post-F tablet ingestion).

154 *Blood plasma:*

155 A 5ml venous blood sample was collected after overnight fasting, prior to taking breakfast (Baseline,
156 T0). An additional four blood samples (5ml/sample) were then collected at 30, 45, 60 and 90 minutes
157 after ingestion of the F-tablet, providing samples T1 to T4.

158 2.4 Analytical Procedure

159 F concentration ($\mu\text{g/ml}$) of urine samples was measured directly after adding total ionic strength
160 adjustment buffer III (Orion Research) to standards and samples, using a F-ion-selective electrode (F-
161 ISE, Model Orion 9609BNWP, Thermo Scientific, USA) coupled to a potentiometer (Model 720A+).
162 F concentrations in plasma (ng/ml) and breakfast items ($\mu\text{g/g}$) were measured, in triplicate, by a
163 hexamethyldisiloxane (HMDS)-facilitated diffusion method (Taves, 1968) which has been previously
164 reported in detail (Martínez-Mier *et al.*, 2011). In summary, 1 ml H_2SO_4 saturated with HMDS was
165 added to 1 ml of sample (and standards) in a petri-dish and left at room temperature to diffuse
166 overnight. An alkaline solution (50 μl of NaOH (0.05N), placed as 5 drops on the inside of the dish
167 lid), was used to trap the released F. After a minimum of 16h diffusion, the NaOH drops were
168 combined as a single drop and 20 μl acetic acid (0.20N) added. The F-ISE electrode was then placed
169 in contact with the combined solution and the mV reading recorded. A calibration curve was used to
170 calculate F concentration of the sample.

171 The reliability of the methods used was specifically confirmed by re-analysis of a minimum 10% of
172 samples. All sample analysis and re-analysis was conducted in triplicate.

173 1.2.5 Data handling and analysis

174 *Urine:*

175 Urinary F excretion (UFE) in each individual time-controlled urine sample was calculated by
176 multiplying the F concentration ($\mu\text{g/ml}$) of the urine sample by its corresponding volume (ml).
177 Baseline-adjusted UFE was calculated by subtracting the baseline UFE from the UFE of each sample.
178 The sums of the amount of F excreted in urine for the periods during and after each experimental
179 session for each participant were used to calculate the total post-F tablet UFE (Periods 2-4 inclusive:
180 representing a 14h period).

181 The UFE rate ($\mu\text{g/h}$) for each given time period was calculated by dividing UFE for each time period
182 by the duration of the corresponding collection period (h).

183 Overall relative UFE (%) was calculated by dividing the baseline-adjusted UFE (μg) for a given time
184 period by the ingested F dose (i.e. 1 mg=1000 μg) multiplied by 100.

185 *Plasma:*

186 Baseline-adjusted plasma F concentration (ngF/ml) was calculated by subtracting the baseline plasma
187 F concentration from the F concentration in each plasma sample.

188 Maximum F concentration (C_{max}) was calculated using the mean maximum baseline-adjusted plasma
189 F concentration following F dose. Lag time to maximum F concentration (T_{max}) was estimated using
190 graphs plotting plasma F concentration against time. Area under the curve (AUC) (ng/min/ml) was
191 calculated using the following equation:

$$192 \text{ AUC} = \sum_{i=0}^{n-1} 0.5(c_i + c_{i+1})(t_{i+1} - t_i), \text{ where:}$$

193 (t) is the number of minutes after F dose - the first time point is time 0 and (Ci) is the value of C at
194 time t_i .

195

196 2.6 Statistical Analysis

197 Descriptive data are presented and statistically significant differences among groups were initially
198 detected using repeated measures ANOVA and further investigated using a post-hoc test (Tukey).
199 Statistical significance was set at $\alpha < 0.05$ and all analysis performed using SPSS version 22.

200

201 **3 Results:**

202 All those invited participated and eight participants (4 males and 4 females) took part in the study.

203 The mean (SD) age, height, weight and BMI for females were: 23.7 (7.2) years, 165.5 (3.5) cm, 64.2
204 (2.5) kg and 23.5 (1.6) kg/m²; and for males were: 25.0 (6.0) years, 176.2 (6.0) cm, 74.2 (9.9) kg and
205 23.7 (2.1) kg/m², respectively.

206 3.1 Accuracy of the analytical method

207 The accuracy of the analytical method was confirmed by comparing the analysis and re-analysis
208 measurements. The results showed no statistically significant differences between the two sets of
209 measurements. The mean (SD) difference for urine samples was 0.009 (0.002) mgF/l (n=16) and for
210 plasma samples was 0.004 (0.001) ngF/ml (n=20).

211 3.2 Comparison of control (no exercise) and the three different exercise intensities

212 Mean (SD) exercise loads for light, moderate and vigorous exercise intensities were 62.5 (37.5), 87.5
213 (37.5) and 112.5 (37.5) Watts for females and 68.7 (37.0), 106.2 (37.0) and 137.5 (37.5) Watts for
214 males, respectively. Mean (SD) maximum heart rates (HR) were 176.0 (12.6) and 160.7 (25.7) bpm
215 and mean RPEs (Rate of Perceived Exertion) at the end of the VO_{2 max} test were 4.7 (0.8) and 7.7 (1.3)
216 in females and males, respectively.

217 Mean (SD) plasma F concentrations, during the control and exercise sessions, according to the
218 different time periods are presented in Table 1 and the pharmacokinetic variables in Table 2.

219 Overall, a total of 32 experimental sessions were undertaken by the eight participants. Mean (SD)
220 baseline fasting plasma F concentration was 31.80 (26.2) ng/ml. Mean baseline-adjusted plasma F
221 concentrations across the 90 minutes post-F ingestion for all experimental sessions are shown in
222 Figure 2.

223 All experimental sessions followed a similar trend in plasma F concentration, peaking between 30 to
224 60 minutes post-F ingestion with a T_{max} ranging from 43 min for light exercise to 50 and 51 min for
225 control and vigorous exercise, respectively. The highest C_{max} was found for moderate exercise
226 (226.2 ngF/ml) followed by light (105.6 ngF/ml), vigorous exercise (94.2 ngF/ml) and control (27.0
227 ngF/ml). AUC_(0-90min) ranged from 15058 ngF/min/ml for moderate exercise to 1474 ngF/min/ml for
228 control.

229 Repeated measures analysis of variation (ANOVA) showed no statistically significant difference in
230 T_{max} among all sessions, whereas C_{max} for moderate exercise was statistically significantly higher
231 compared to no (p < 0.001), light (p = 0.016) and vigorous (p = 0.008) exercise. AUC_(0-90min) was also
232 statistically significant higher at moderate exercise intensity compared to no (p < 0.001), light (p =
233 0.004) and vigorous (p = 0.001) exercise.

234 The mean (SD) UFE at baseline was 109.2 (100.7) µgF for the total of 32 experimental sessions
235 undertaken overall by the eight participants. Mean (SD) UFEs for the different time periods during the
236 control and exercise sessions are presented in Table 3.

237 No statistically significant differences in UFE were found between the no exercise and three different
238 exercise intensities for any individual time period, nor for total post-F tablet period (i.e. 0-14h post-F
239 tablet ingestion).

240 Mean baseline-adjusted UFE rates across the 4 time-controlled periods of urine collection are shown
241 in Figure 3.

242 Light, moderate and vigorous intensity exercise resulted in lower mean baseline-adjusted UFE rates
243 over the 0-3h post-F tablet period (light 41.1, moderate 25.6 and vigorous 35.3µgF/h,) in comparison
244 with no exercise (62.6µgF/h); however, the differences were not statistically significant. Furthermore,

245 there were no statistically significant differences in baseline-adjusted UFE rates among different
246 exercise intensities (including no exercise) for any individual time period.

247 Mean overall relative UFE (i.e. proportion of ingested F dose excreted in urine) for each time period
248 was 21% for 0-3h post-F tablet, 20% for 3-8h post-F tablet and 16% for 8-14h post-F tablet, with an
249 overall relative UFE of 59% for 0-14h post-F tablet.

250

251 **4 Discussion:**

252 This study provides the first data on the effects of exercise on F pharmacokinetics in healthy adults.
253 The results suggest that moderate exercise may result in higher F absorption and consequently higher
254 body F retention. These observations could be particularly important in communities with fluoridation
255 programmes such as school-based milk fluoridation where children consume fluoridated milk just
256 before mid-morning playtime (e.g. in UK school milk fluoridation programmes).

257 The mean T_{max} (50 min) and $AUC_{(0-90min)}$ (1474 ngF/min/ml) reported in our study for the control
258 session (no exercise, received 1mgF tablet) were within the corresponding ranges of 43.1-56.6 min
259 and 752-1562 ngF/min/ml, respectively, reported for 21-35 year old English adults given a F dose of
260 0.5 mg (500 ml of fluoridated water containing almost 1mgF/L) (Maguire *et al.*, 2005). However, the
261 mean C_{max} (27.0 ng/ml) for the no exercise (control) session in our study was higher than the
262 corresponding range of 9.2-19.0 ng/ml reported for English adults (Maguire *et al.*, 2005). Since F
263 dose is an important factor influencing F pharmacokinetics, the observed higher pharmacokinetic
264 parameters in our study, compared to the study by Maguire et al (2005) could be explained by the
265 larger amounts of F ingested by participants in our study.

266 Our study found a non-statistically significant trend for an overall lower UFE with greater exercise
267 intensity. However, the overall mean plasma F concentrations at different time points, as well as
268 C_{max} and $AUC_{(0-90min)}$ were higher for the exercise sessions compared to the no exercise (control)
269 session (Tables 1 and 2; Figure 1). These findings imply that exercise could affect the
270 pharmacokinetics of F, i.e. increasing F absorption but decreasing F excretion. However, the

271 mechanisms by which exercise could alter F metabolism remain unclear. The increase in cardiac
272 output and consequently muscle and skeletal blood flow following exercise may lead to an increase in
273 the rate of F absorption and body distribution to muscles and bones. Additionally, exercise could
274 affect renal clearance of F from kidneys in two ways: (a) increase the activity of sympathetic nervous
275 system, resulting in vasoconstriction within the kidney which would then reduce renal blood flow and
276 glomerular filtration rate (GFR); and (b) increase production of lactic acid by muscle which would
277 increase the renal reabsorption of F (Whitford, 1996; Buzalaf and Whitford, 2011). These changes
278 would further lessen the renal excretion of F but tend to increase levels of F in plasma.

279 Our study found no statistically significant difference in T_{max} among different intensities of exercise
280 including no exercise (control). However, our study showed that the mean values for C_{max} and
281 AUC_(0-90min) were statistically significantly higher for moderate exercise compared with light and
282 vigorous exercise as well when compared with no exercise (control). A study with nine adults
283 (Zohoori *et al.*, 2015) also reported higher plasma F concentrations, although not statistically
284 significant, for moderate intensity exercise compared with control, light and vigorous exercise.

285 Gastric emptying has been shown to increase with increasing exercise intensities up to 65% VO₂ max
286 (moderate intensity), but it decreases above an intensity of 75% VO₂ max (vigorous intensity)
287 (Neufer, 1989). Cardiac output following an increased work rate increases in an almost linear manner
288 to meet the increasing oxygen demand but only up to the point where maximal capacity is reached
289 (Manley 1996). This may explain the higher mean plasma F concentration, C_{max} and AUC_(0-90min) for
290 the moderate compared to vigorous exercise as participants may have reached their maximum cardiac
291 output when exercising at vigorous intensity. Future studies are therefore needed to include
292 interventions where participants undertake an exercise routine at different intensities (light, moderate
293 and vigorous), for a prolonged period of time.

294 Urine is the major excretion route for systemically absorbed bioavailable F, with the majority of an
295 ingested F dose appearing in the urine within the first three hours (Zipkin and Leone, 1957). Our
296 study showed that, on average, 59% of daily intake of F was excreted in urine, over a 24h period,
297 which is in agreement with the suggested corresponding figure of 60% for healthy adults (Buzalaf and

308 Whitford, 2011). Our study also found that, on average, 21% of ingested F dose was excreted in urine
309 during the first three hours following F ingestion, corresponding to the value of 20% reported for
310 healthy adults (Zipkin and Leone, 1957).

311 Our study showed a lower, although not statistically significant, UFE rate over the first 3h period for
312 moderate exercise compared with light and vigorous exercise as well as when compared with no
313 exercise (Table 3). However, the UFE rate tended to be higher over the 3-14h period for moderate
314 exercise compared with other exercise intensities including no exercise. These findings indicate that
315 moderate exercise may lead to a delay in urinary F excretion in adults. The lower UFE rate over the
316 first 3h period could be explained by the increased production of lactic acid, leading to a more acidic
317 urine and consequently resulting in a higher proportion of ingested F being reabsorbed (i.e. lower
318 urinary F excretion). In addition, it is known that a steady-state relationship exists between plasma F
319 levels and the hydration shell of the bone crystallites (Rao *et al.*, 1995). Thus, another possibility is
320 that moderate exercise increases the absorption of F, thus augmenting plasma F levels, which in turn
321 would increase F uptake in the hydration shell of the bone crystallites. As plasma F levels start to
322 decrease after the peak is reached, then F present in the hydration shell of the bone crystallites is
323 released back into plasma and excreted in urine over time.

324 In order to reduce dental caries in children, public health initiatives such as school fluoridated milk
325 programmes have been rolled out across schools in some counties including the UK (Banoczy *et al.*,
326 2009). However, previous studies have indicated that current UK milk fluoridation programmes do
327 not provide adequate protection for the prevention of dental caries (Ketley and Lennon, 2000). It has
328 been reported that increasing the school milk F dose from 0.5mg to 0.9mg per 189ml, in the UK, may
329 still be too low to achieve the World Health Organisation recommended UFE concomitant with
330 optimal F exposure for children aged < 6y (World Health Organization, 2014). In the UK, fluoridated
331 milk, is often provided to schoolchildren during their mid- morning break before undergoing physical
332 activity. In addition, UK children's physical activity levels during break have been reported to be
333 predominantly moderate (Powell *et al.*, 2016). According to our findings, the low UFE observed
334 (Maguire *et al.*, 2013) during monitoring of fluoridated milk programmes may therefore be related to

325 the effect of moderate physical activity, that children undertake during their breaks, on F absorption
326 and excretion. F concentrations in blood and urine have been shown not to be influenced by sex
327 (Torra *et al.*, 1998; Del Carmen *et al.*, 2016) However, due to the possible different physiological
328 responses following exercise in children compared to adults, as well as females compared to males,
329 further work is required to determine the effects of exercise on F metabolism in young children and
330 different sexes. These findings can help inform the evidence base for stakeholders and decision
331 makers in dental public health as well as health professionals who may wish to review F dose and
332 time of administration in different fluoridation programmes.

333 The main limitations of our study are: (i) the sample size; although the number of participants in this
334 study are comparable with other similar studies in humans (Zohoori *et al.*, 2015) and animals (Whitford
335 *et al.*, 1988; Lombarte *et al.*, 2013); (ii) the F dose; which was based on the optimal F concentration of
336 drinking water of 1mg/l, and; (iii) the inclusion of only one age group (young adults). Since the peak
337 plasma and bone F concentrations are directly related to both the age of the individual and F intakes,
338 any extrapolation of the study findings to other age groups should be made with caution.

339 Our study also indicated large variation in pharmacokinetic variables between individuals. A study by
340 Ekstrand [Ekstrand, 1978] with a family of five, aged 10 to 38 years old, who ate together and received
341 a water supply with 9.6 ppm F, showed a large variation in plasma F concentration between family
342 members and a much greater within-individual variation during the day (e.g. 40-110 ng/ml for an adult
343 family member). Some of the relatively wide variation in pharmacokinetic variables between
344 participants might be explained by between-individual differences in physiological variables such as
345 volume and pH of gastric secretions, gastro-intestinal motility, plasma volume, and urinary pH.

346 In conclusion, this human experimental study adds to the understanding of the effects of exercise on F
347 metabolism. The findings suggest that moderate exercise may increase the fraction of ingested F
348 absorbed systemically and therefore available to produce a biological effect. In addition, moderate
349 exercise may have a tendency to delay the excretion of F in urine.

350

351 **References:**

- 352 Amaral, S.L., Azevedo, L.B., Buzalaf, M.A.R., Fabricio, M.F., Fernandes, M.S., Valentine, R.A.,
353 Maguire, A. and Zohoori, F.V. (2018) 'Effect of chronic exercise on fluoride metabolism in fluorosis-
354 susceptible mice exposed to high fluoride', *Sci Rep*, 8(1), p. 3211.
- 355 American College of Sports Medicine (2007) *ACSM's Health/Fitness Facility Standards and*
356 *Guidelines*, 4E. Human Kinetics.
- 357 Banoczy, J., Petersen, P.E. and Rugg-Gunn, A. (eds.) (2009) *Milk fluoridation for the prevention of*
358 *dental caries*. Geneva: World Health Organization.
- 359 Beaver, W.L., Wasserman, K. and Whipp, B.J. (1986) 'A New Method for Detecting Anaerobic
360 Threshold by Gas-Exchange', *J. Appl. Physiol.*, 60(6), pp. 2020-2027.
- 361 Buzalaf, M.A. and Whitford, G.M. (2011) 'Fluoride Metabolism', in Buzalaf, M.A. (ed.) *Fluoride and*
362 *the Oral Environment*. Basel, Switzerland: Karger.
- 363 Buzalaf, M.A.R. (2018) 'Review of Fluoride Intake and Appropriateness of Current Guidelines', *Adv*
364 *Dent Res*, 29(2), pp. 157-166.
- 365 Craig, C.L., Marshall, A.L., Sjostrom, M., Bauman, A.E., Booth, M.L., Ainsworth, B.E., Pratt, M.,
366 Ekelund, U., Yngve, A., Sallis, J.F. and Oja, P. (2003) 'International physical activity questionnaire:
367 12-country reliability and validity', *Med Sci Sport Exer*, 35(8), pp. 1381-1395.
- 368 Evans, C.H. and White, R.D. (2009) *Exercise testing for primary care and sports medicine physicians*.
369 1st edn. New York: Springer.
- 370 Ketley, C.E. and Lennon, M.A. (2000) 'Urinary fluoride excretion in children drinking fluoridated
371 school milk', *Int. J. Paediatr. Dent*, 10(4), pp. 260-270.
- 372 Lombarte, M., Fina, B.L., Lupo, M., Buzalaf, M.A. and Rigalli, A. (2013) 'Physical exercise
373 ameliorates the toxic effect of fluoride on the insulin-glucose system', *J Endocrinol*, 218(1), pp. 99-
374 103.

375 Maguire, A., Walls, R., Steen, N., Teasdale, L., Landes, D., Omid, N., Moynihan, P. and Zohoori,
376 F.V. (2013) 'Urinary Fluoride Excretion in 6- to 7-Year-Olds Ingesting Milk Containing 0.5 or 0.9 mg
377 Fluoride', *Caries Res*, 47(4), pp. 291-298.

378 Maguire, A., Zohouri, F.V., Mathers, J.C., Steen, I.N., Hindmarch, P.N. and Moynihan, P.J. (2005)
379 'Bioavailability of Fluoride in Drinking Water: a Human Experimental Study', *J Dent Res*, 84(11), pp.
380 989-993.

381 Martínez-Mier, E.A., Cury J.A., Heilman J.R. , Katz B.P., Levy S.M., Li Y., Maguire A., Margineda
382 J., O'Mullane D., Phantumvanit P., Soto-Rojas A.E., Stookey G.K., Villa A., Wefel J.S., Whelton H. ,
383 Whitford G.M., Zero D.T., Zhang W. and Zohouri, V. (2011) 'Development of gold standard ion-
384 selective electrode-based methods for fluoride analysis ', *Caries Res*, 45, pp. 3-12.

385 Neuffer, P.D. (1989) 'The Effect of Detraining and Reduced Training on the Physiological Adaptations
386 to Aerobic Exercise Training', *Sport Med*, 8(5), pp. 302-320.

387 Noble, B.J., Borg, G.A.V., Jacobs, I., Ceci, R. and Kaiser, P. (1983) 'A Category-Ratio Perceived
388 Exertion Scale - Relationship to Blood and Muscle Lactates and Heart-Rate', *Med Sci Sport Exer*,
389 15(6), pp. 523-528.

390 Palmer, C. and Wolfe, S.H. (2005) 'Position of the American Dietetic Association: the impact of
391 fluoride on health', *J Am Diet Assoc*, 105(10), pp. 1620-8.

392 Powell, E., Woodfield, L.A. and Nevill, A.A.M. (2016) 'Children's physical activity levels during
393 primary school break times: A quantitative and qualitative research design', *Eur. Phys. Educ. Rev.*,
394 22(1), pp. 82-98.

395 Rao, H.V., Beliles, R.P., Whitford, G.M. and Turner, C.H. (1995) 'A physiologically based
396 pharmacokinetic model for fluoride uptake by bone', *Regulatory Toxicology and Pharmacology*,
397 22(1), pp. 30-42.

398 Schwab, P. and Scalapino, K. (2011) 'Exercise for bone health: rationale and prescription', *Curr Opin*
399 *Rheumatol*, 23(2), pp. 137-41.

400 Taves, D.R. (1968) 'Separation of fluoride by rapid diffusion using hexamethyldisiloxane', *Talanta*,
401 15(9), pp. 969-974.

402 ten Cate, J.M. and Buzalaf, M.A.R. (2019) 'Fluoride Mode of Action: Once There Was an Observant
403 Dentist ', *J Dent Res*, 98(7), pp. 725-730.

404 Torra, M., Rodamilans, M. and J Corbella,J. (1998) Serum and urine fluoride concentration:
405 Relationships to age, sex and renal function in a non-fluoridated population, *Sci Total Environ*, 220
406 (1), 81-5. doi.org/10.1016/S0048-9697(98)00248-4.

407 Del Carmen, A.F., Javier, F.H., Aline, C.C. (2016)Dental fluorosis, fluoride in urine, and nutritional
408 status in adolescent students living in the rural areas of Guanajuato, Mexico. *J Int Soc Prev
409 Community Dent*. 6(6):517-522. doi:10.4103/2231-0762.195510

410 Whitford, G.M. (1996) *The Metabolism and Toxicity of Fluoride*. Basel: Karger.

411 Whitford, G.M., Birdsong-Whitford, N.L. and Lowe, S.R. (1988) 'Fluoride pharmacokinetics: effect
412 of light exercise in rats.', *Caries Res*, 22, p. Abs 106.

413 Willems, H.M.E., van den Heuvel, E.G.H.M., Schoemaker, R.J.W., Klein-Nulend, J. and Bakker,
414 A.D. (2017) 'Diet and Exercise: a Match Made in Bone', *Current Osteoporosis Reports*, 15(6), pp.
415 555-563.

416 World Health Organization (2014) *Basic methods for assessing renal fluoride excretion in community
417 prevention programmes for oral health* Geneva, Switzerland: World Health Organization,.

418 Yu, H., Jiang, N., Yu, X., Zhao, Z., Zhang, X. and Xu, H. (2018) 'The role of TGFbeta receptor 1-
419 smad3 signaling in regulating the osteoclastic mode affected by fluoride', *Toxicology*, 393, pp. 73-82.

420 Zipkin, I. and Leone, N.C. (1957) 'Rate of urinary fluoride output in normal adults', *Am J Public
421 Health*, 47(7), pp. 848-51.

422 Zohoori, F.V., Innerd, A., Azevedo, L.B., Whitford, G.M. and Maguire, A. (2015) 'Effect of exercise
423 on fluoride metabolism in adult humans: a pilot study', *Sci Rep*, 5.

424

425

426 **Competing interests:**

427 The authors declare no potential conflicts of interest with respect to the authorship and/or publication
428 of this article.

429 **Author contributions:**

430 FVZ and MM conceived the study; FVZ, MM, and LBA designed the study; MM collected and
431 analyzed the samples; FVZ supervised the project with help from LBA; FVZ, and MM analyzed the
432 data and LBA, AM, and MB contributed to the interpretation of the results; FVZ, MM and AM took
433 the lead in writing the manuscript. All authors read, provided critical feedback and approved the
434 submitted paper.

435 **Acknowledgement**

436 This study was supported by an internal grant from Teesside University as well as an external grant
437 from The Borrow Foundation.

438

439 **Table 1.** Mean (SD) plasma fluoride (F) concentrations (ngF/ml), during experimental sessions; no
440 exercise (control), light, moderate and vigorous exercise.

441

| Post-F ingestion plasma collection time | Exercise intensity | | | |
|---|--------------------------|---------------|---------------|--------------|
| | No exercise (Control) | Light | Moderate | Vigorous |
| 30 minutes (T1) | 20.4 (14.7) | 150.9 (54.9) | 241.3 (130.1) | 93.8 (36.7) |
| 45 minutes (T2) | 29.6 (16.6) | 147.9 (77.5) | 263.1 (113.6) | 136.9 (59.8) |
| 60 minutes (T3) | 33.6 (26.9) | 134.6 (104.7) | 238.7 (74.1) | 127.5 (24.9) |
| 90 minutes (T4) | 16.6 (12.5) | 110.0 (98.7) | 205.2 (83.3) | 111.4 (42.9) |

442

443

444 **Table 2.** Mean (SD) pharmacokinetic parameters for plasma following ingestion of fluoride (F) tablet
445 (1.0 mg F) by exercise intensity.

446

| Pharmacokinetic parameters for plasma F | Exercise intensity | | | |
|---|--------------------------|--------------|---------------|-------------|
| | No exercise (Control) | Light | Moderate | Vigorous |
| T_{\max} (min) ^a | 50.3 (11.0) | 42.9 (13.5) | 45.9 (10.5) | 51.3 (10.9) |
| C_{\max} (ngF/ml) ^b | 27.0 (24.3) | 105.6 (41.7) | 226.2 (115.6) | 94.2 (58.1) |
| AUC _(0-90min) (ngF.min.ml ⁻¹) ^c | 1474 (939) | 6920 (3506) | 15058 (6596) | 5542 (2264) |

447

448 a) Lag time to maximum F concentration

449 b) Maximum F concentration

450 c) Area under the curve

451

452 **Table 3.** Mean (SD) urinary fluoride excretion (UFE; μgF) for different time-controlled periods
453 during experimental sessions for no exercise (control), light, moderate and vigorous exercise.

454

| UFE (Time period) | Exercise intensity | | | |
|--------------------------------------|--------------------------|---------------|---------------|---------------|
| | No exercise (Control) | Light | Moderate | Vigorous |
| UFE _{0-3h} (9:00 – 12:00) | 302.7 (354.4) | 222.3 (64.0) | 119.4 (132.4) | 213.5 (100.0) |
| UFE _{3-8h} (12:00 – 17:00) | 214.0 (167.8) | 207.4 (116.4) | 242.9 (100.3) | 130.8 (74.1) |
| UFE _{8-14h} (17:00 – 23:00) | 130.3 (122.2) | 196.0 (206.7) | 207.8 (134.7) | 124.5 (52.5) |
| UFE ₀₋₁₄ (09:00 – 23:00) | 638.8 (565.5) | 718.7 (296.8) | 574.6 (281.1) | 450.5 (206.1) |

455

456

457

458

459

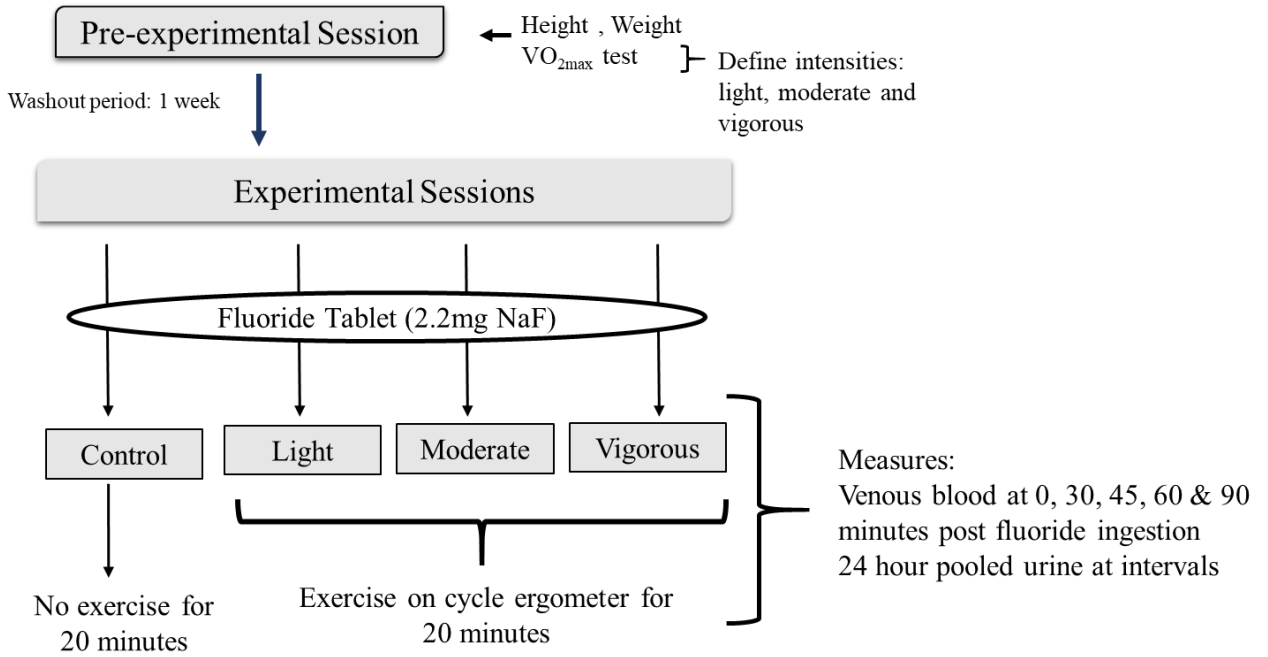
460

461

462

463

464



465

466

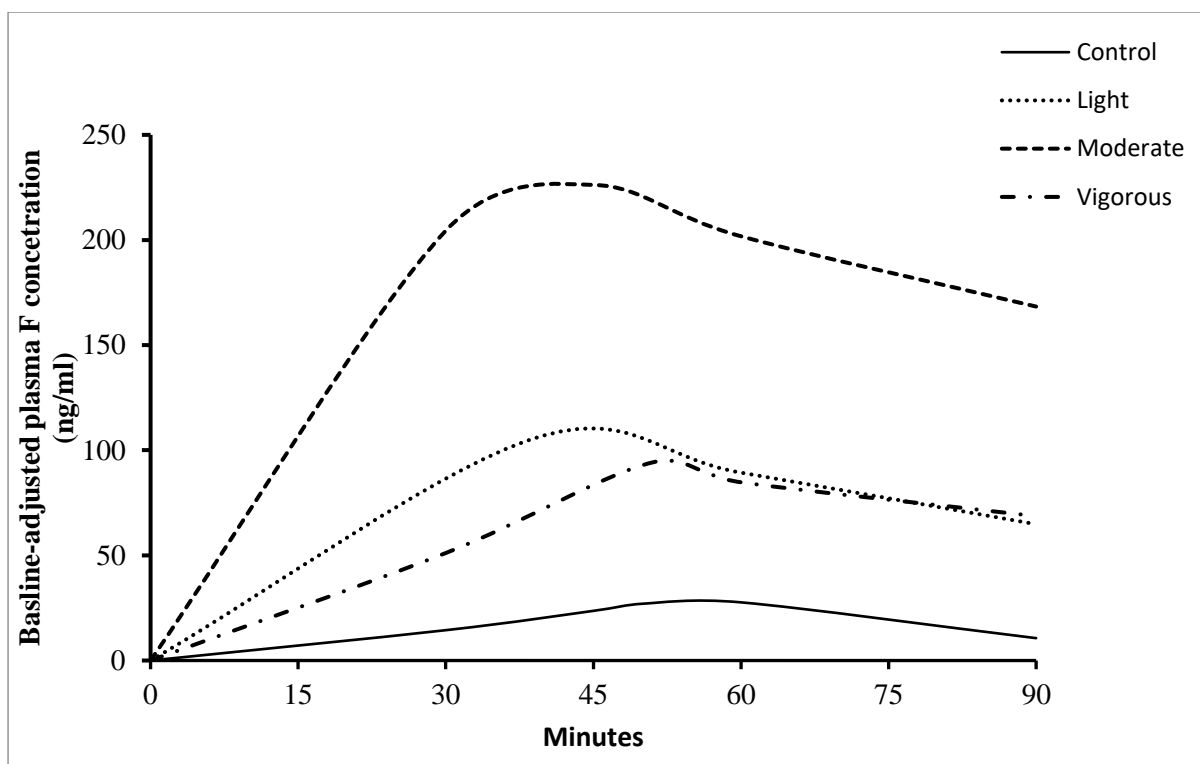
467 **Figure 1.** Experimental procedure and sample collection

468

469

470

471



472

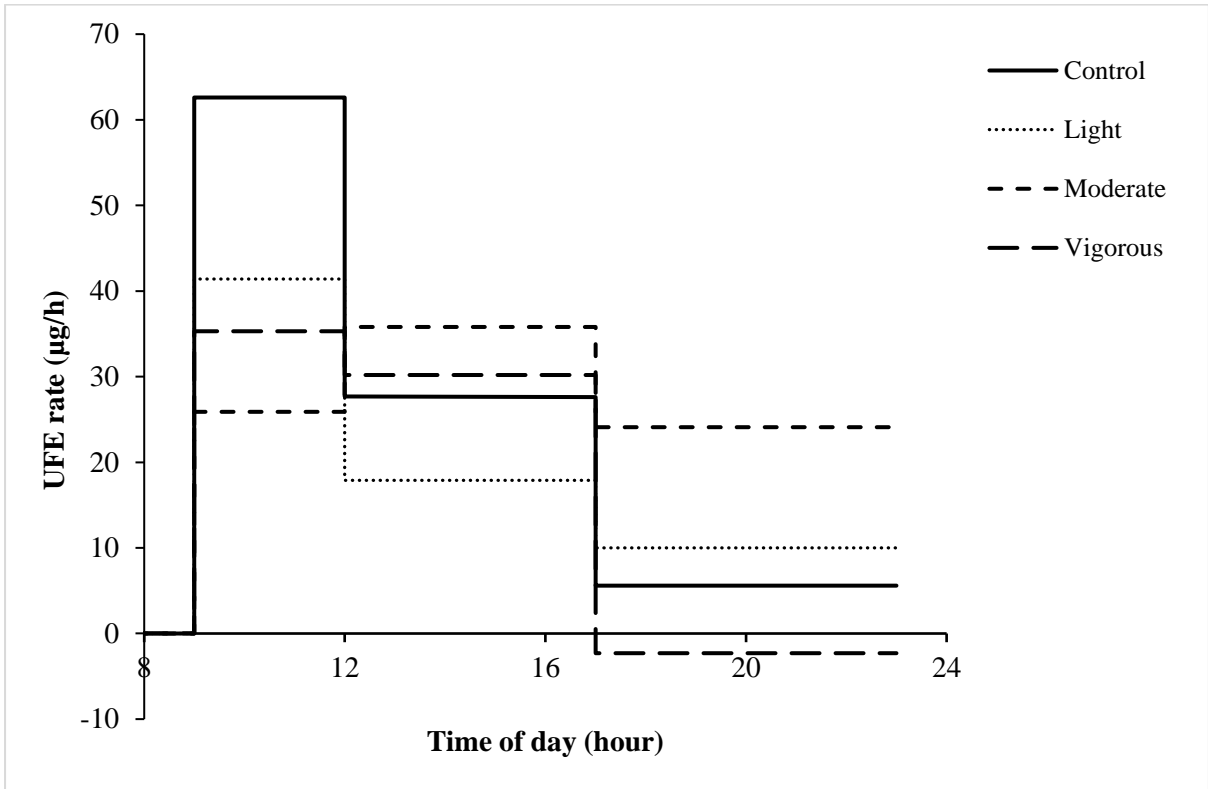
473

474 **Figure 2.** Baseline-adjusted plasma F concentration (ng/ml) over the 0 - 90 minute post F ingestion

475 period.

476

477



479

480

481 **Figure 3.** Mean baseline-adjusted UFE rate (µg/h) across the 4 time-controlled periods of collection
482 according to exercise intensity; no exercise (control (blue line)), light (green line), moderate (brown
483 line) and vigorous exercise (yellow line)

484

485

486