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Daily variations in human plasma fluoride concentrations

Vanessa E.S. Cardoso [a], Gary M. Whitford [b], Hiroshi Aoyama [c], Marilia A.R. Buzalaf [d,*]

[a] Institute of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes, 748, CEP 05508-900, São Paulo-SP, Brazil
[b] Department of Oral Biology, School of Dentistry, Medical College of Georgia, Augusta, 30912-1129, GA, USA
[c] Department of Biochemistry, University of Campinas, R Monteiro Lobato, 255, CEP 13083-862, Campinas-SP, Brazil
[d] Department of Biological Sciences, Bauru Dental School, University of São Paulo, Al. Octávio Pinheiro Brisolla, 9-75, CEP 17012-901, Bauru-SP, Brazil

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ABSTRACT

This study investigated the variations in human plasma fluoride concentrations ([F]) and sought to determine the causes. Five subjects (27–33 years old) received a low-F diet during the 5 days of the study. Plasma samples and urine were collected every 3 h from 8 a.m. to 8 p.m. F, PTH, Ca and P were analyzed with the electrode, by chemiluminescence, AAS and colorimetry, respectively. A trend for the plasma [F] was found. The peak [F], 0.55 ± 0.11 μmol L^{-1}, occurred at 11 a.m. and the lowest [F], 0.50 ± 0.06 μmol L^{-1} occurred between 5 and 8 p.m. Plasma [F] were positively correlated with urinary F excretion rates and with serum PTH levels, but not with the Ca or P levels. Serum PTH levels were positively correlated with urinary F excretion rates and negatively correlated with plasma Ca. The results suggest that the renal system seems to control the daily fluctuations in plasma [F].

1. Introduction

Variations in plasma fluoride concentrations ([F]) have been studied under various conditions. In the 1960s and 1970s plasma [F] were directly related to the fluoride concentration in drinking water [1,2]. Nowadays this relationship would be less apparent due to the fluoride intake from multiple sources. It has been established that plasma fluoride levels in humans vary during the day [2,3] due to the normal patterns of daily fluoride intake [4].

The possible existence of a biological rhythm for the plasma [F] not related to intake was raised based on the calcium biological rhythms [4–6]. Variations during the day in plasma concentrations of these ions are partly attributed to the balance between bone formation and resorption, influenced by the hormones that act on bone. Nearly all (>99%) of fluoride retained in the body is associated with the skeleton. Thus, we hypothesized that plasma fluoride levels would show a biological rhythm similar to and coordinated with the calcium rhythm.

A biological rhythm for plasma F has been observed in dogs [4]. Peak plasma [F] was found at 9 a.m., while the trough was observed at 9 p.m. In a subsequent study, calcium and phosphate rhythms were also found [4]. These were coincident, while the fluoride rhythm was 180° out of phase. Thus, the aim of this study was to investigate the variations in human plasma [F] and to determine the physiological systems involved in its regulation.

2. Results

Fig. 1 shows the schedule for when blood and urine were collected and meals were eaten. Mean dietary F intakes (±SD) with breakfast, lunch, afternoon snack and dinner were 0.04 ± 0.02, 0.07 ± 0.03, 0.02 ± 0.01 and 0.10 ± 0.051 mg, respectively.

Table 1 shows plasma [F] (μmol L^{-1}) as a function of the time of blood collection for each subject, individually, during the 5 days of the study.

Fig. 2A shows that fluctuations in plasma [F] were found ($p = 0.0029$). The average peak, 0.55 μmol L^{-1}, occurred at 11 a.m. and the trough, 0.50 μmol L^{-1} occurred 6 h later, at 5 p.m. and was maintained until 8 p.m. ($p < 0.05$). The plasma [F] for day 1 was higher than those on subsequent days (Fig. 2A). For day 1, significant differences in plasma [F] were found at 8 a.m. when compared to 5 and 8 p.m. ($p < 0.001$), and for 11 a.m. when compared to 5 and 8 p.m. ($p < 0.05$). For day 2 to day 5, significant differences in plasma [F] were found at 11 a.m. when compared to

* Corresponding author. Present address: Department of Biological Sciences (Biochemistry), Bauru Dental School, University of São Paulo, Bauru, SP. Al. Octávio Pinheiro Brisolla, 9-75, 17012-901 Brazil. Tel.: +55 14 3235 8346; fax: +55 14 3227 1486.
E-mail address: mbuzalaf@fob.usp.br (Marilia A.R. Buzalaf).

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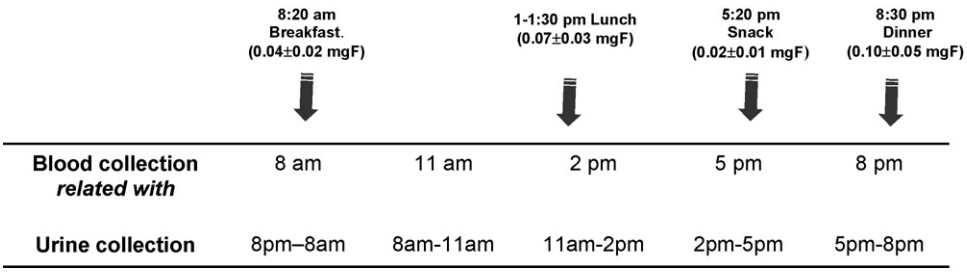

Fig. 1. Experimental design. Times of blood and urinary collections and times and fluoride concentrations of the meals. $n = 5$.

8 p.m. ($p < 0.05$). Despite the differences in plasma [F] between the day 1 and the subsequent days, a similar profile was apparent. Plasma [F] for the five individual subjects, V1–V5, are shown in Fig. 2B–F, respectively.

As seen in Fig. 3A, serum PTH concentration also showed variations during the day ($p = 0.0004$). Samples collected at 2 and 8 p.m. had higher PTH levels when compared to those collected at 8 and 11 a.m ($p < 0.05$). Fig. 3B shows that plasma calcium concentration also had fluctuations ($p = 0.0024$). They increased at 11 a.m. and decreased during the day ($p < 0.05$). Fig. 3C shows that slight fluctuations in plasma phosphorus concentration were observed. The levels decreased at 11 a.m., returning to the 8 a.m. levels at 5 p.m.

Fig. 4A shows that urinary fluoride excretion also varied as a function of the time of sample collection ($p = 0.026$). Differences in fluoride excretion were found for the 11 a.m. samples when compared to the 5 p.m. samples ($p < 0.05$). Fig. 4B shows that there were differences in urinary flow rate with the time of sample collection ($p = 0.0064$). Differences in urinary flow rate were found for the 8 a.m. samples when compared to the 5 and 8 p.m. samples ($p < 0.05$), and also for the 11 a.m. samples when compared to the 5 p.m. samples ($p < 0.01$).

Table 2 shows the correlation between the variables evaluated. Significant positive correlations were found between plasma [F] and serum PTH concentration; plasma [F] and the urinary fluoride excretion; serum PTH concentration and the urinary excretion of fluoride; urinary F excretion and urinary flow rate. Significant negative correlations were found between plasma [F] and urinary flow rate; serum PTH concentration and the plasma calcium concentration. No significant correlations were observed between plasma [F] and plasma calcium or phosphate concentrations.

3. Discussion

As shown in Fig. 2A–F, our findings provide evidence for the fluctuations in plasma [F] in humans during the daytime. The average peak occurred at 11 a.m. and the trough occurred between 5 and 8 p.m. (Fig. 2A). The presence of variations during the day is in agreement with the findings of Whitford [4] for dogs. The main difference was that the average peak plasma [F] in dogs occurred at 9 a.m. and the average trough occurred 12 h later at 9 p.m. These differences in peak and trough times might be explained in part by the fact that the room in which the dogs were housed had a light/dark cycle of 6 a.m. to 6 p.m./6 p.m. to 6 a.m., while exposures to

Table 1
Plasma fluoride concentration (μmol L^{-1}) as a function of the time of blood collection for each subject, individually, during the 5 days of the study.

Subject		Plasma fluoride concentration					
		Day 1	Day 2	Day 3	Day 4	Day 5	Mean ± SD
V1	8 a.m.	0.76	0.44	0.47	0.48	0.45	0.52 ± 0.13
	11 a.m.	0.61	0.49	0.56	0.47	0.51	0.53 ± 0.06
	2 p.m.	0.56	0.44	0.51	0.51	0.52	0.51 ± 0.04
	5 p.m.	0.52	0.46	0.55	0.49	0.48	0.50 ± 0.04
	8 p.m.	0.48	0.43	0.46	0.53	0.42	0.46 ± 0.04
V2	8 a.m.	0.73	0.69	0.64	0.64	0.60	0.66 ± 0.05
	11 a.m.	0.68	0.71	0.66	0.61	0.61	0.65 ± 0.04
	2 p.m.	0.74	0.67	0.62	0.61	0.57	0.64 ± 0.07
	5 p.m.	0.61	0.62	0.64	0.53	0.54	0.59 ± 0.05
	8 p.m.	0.60	0.63	0.63	0.54	0.53	0.59 ± 0.04
V3	8 a.m.	0.55	0.50	0.48	0.44	0.42	0.47 ± 0.05
	11 a.m.	0.67	0.42	0.52	0.43	0.43	0.49 ± 0.11
	2 p.m.	0.52	0.43	0.48	0.47	0.48	0.48 ± 0.03
	5 p.m.	0.44	0.47	0.48	0.40	0.43	0.44 ± 0.03
	8 p.m.	0.48	0.39	0.53	0.47	0.43	0.46 ± 0.05
V4	8 a.m.	0.51	0.45	0.50	0.39	0.40	0.45 ± 0.06
	11 a.m.	0.54	0.54	0.54	0.47	0.50	0.52 ± 0.03
	2 p.m.	0.49	0.55	0.46	0.51	0.58	0.52 ± 0.05
	5 p.m.	0.46	0.53	0.45	0.47	0.54	0.49 ± 0.04
	8 p.m.	0.49	0.52	0.45	0.47	0.50	0.49 ± 0.03
V5	8 a.m.	0.72	0.50	0.55	0.48	0.47	0.54 ± 0.10
	11 a.m.	0.69	0.66	0.52	0.47	0.46	0.56 ± 0.11
	2 p.m.	0.55	0.51	0.49	0.50	0.52	0.51 ± 0.02
	5 p.m.	0.57	0.52	0.45	0.45	0.47	0.49 ± 0.05
	8 p.m.	0.53	0.48	0.45	0.47	0.49	0.48 ± 0.03
Mean ± SD		0.58 ± 0.10	0.52 ± 0.09	0.52 ± 0.07	0.49 ± 0.06	0.49 ± 0.06	

V1, V2 and V3 are female and V4 and V5 are male.

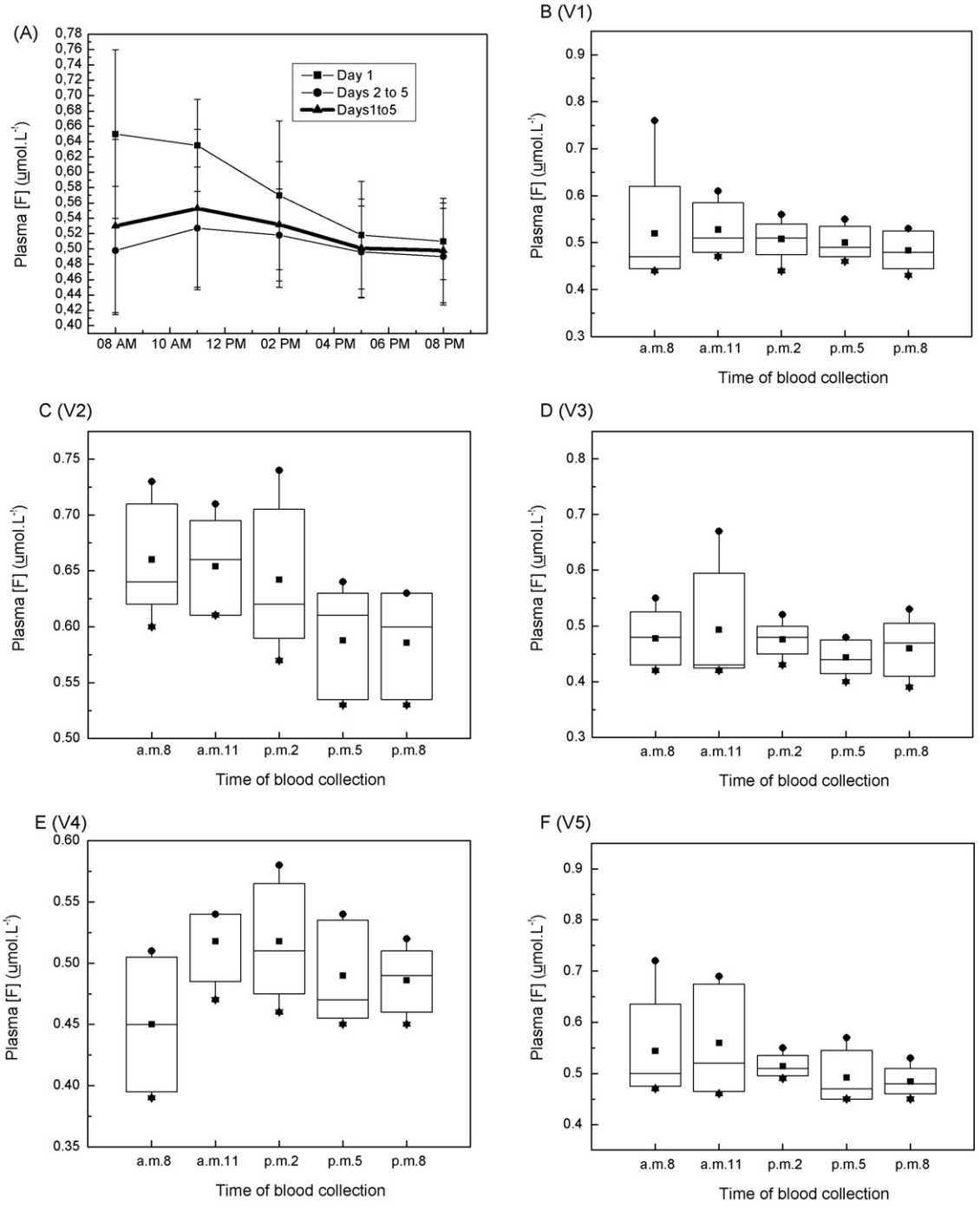

Fig. 2. (A) Plasma fluoride concentration (μmol L^{-1}) for all subjects (n = 5) as a function of the time of blood collection for day 1 (■ symbol), for days 2–5 (● symbol) and for the 5 experimental days (▲ symbol). Data are shown as mean (±SD). (B–F) Box plots of plasma fluoride concentration (μmol L^{-1}) as a function of the time of blood collection for the 5 individual subjects, V1–V5, respectively. Solid squares indicate mean values.

light and dark were not controlled in our study. Biological rhythms for many variables are known to be partially regulated by light/dark cycles [7]. In addition, the dogs were constantly infused intravenously from an ambulatory pump with 0.9 mg F/kg/day and they were fed only once each day at 3 p.m. Routine manipulations in laboratory animals, such as drug administrations, can have important effects on mammalian circadian rhythms [8]. In the present study we controlled the composition of the diet, the time of eating and the schedule of blood collections. If the light/dark cycle and the activities of the subjects had been controlled, then the differences in the profiles of plasma [F] seen among the 5 days of blood collections (Table 1) could have been less pronounced.

We are aware of only one other study in which evidence for the changes in human plasma [F] was sought [9]. Their six subjects (mean age 58 years) had fasted from midnight of the previous day to 3:30 p.m. of the day of the experiment and drank only deionized water. Blood samples were collected from each subject at 8 a.m., 12 noon and 3:30 p.m. The average serum [F] was 1.75, 1.83 and 1.96 μmol L^{-1}, respectively. This gradual upward trend is similar to that of the present study during the morning hours (Fig. 2A).

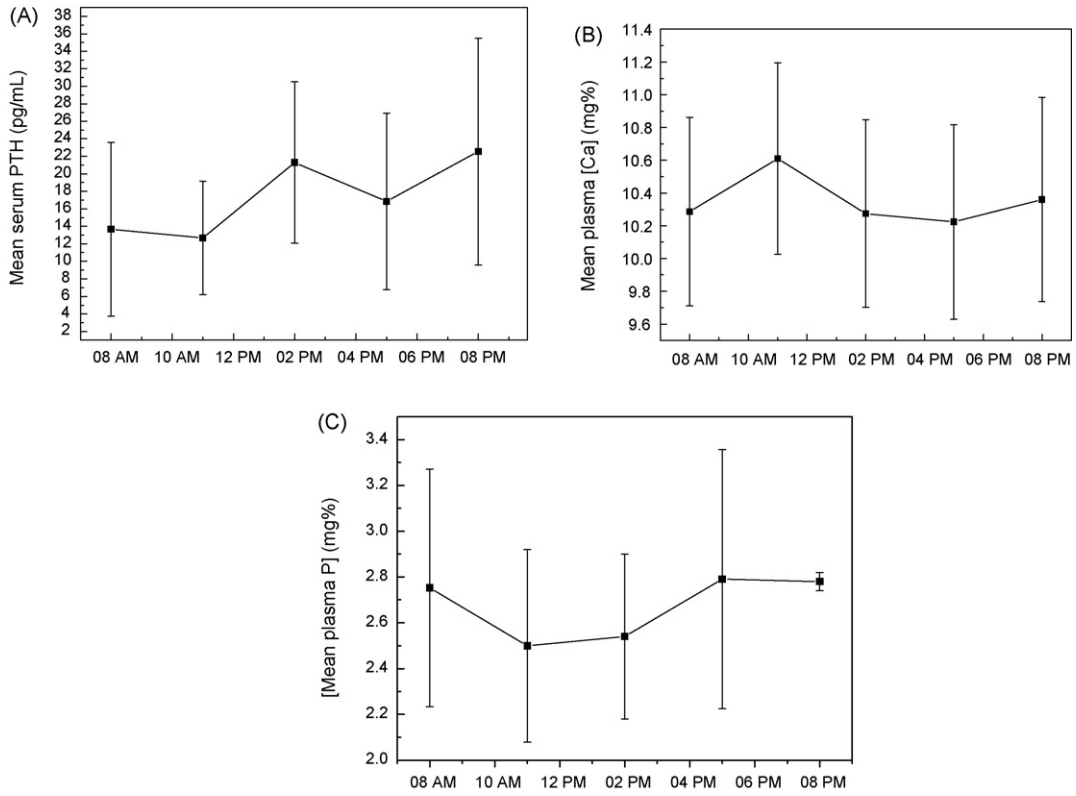

Fig. 3. (A) Serum PTH levels (pg mL^{-1}) as a function of the time of blood collection. (B) Plasma calcium concentrations (mg%) as a function of the time of blood collection. (C) Plasma phosphorus levels (mg%) as a function of the time of blood collection. Data are shown as mean (±SD). (n = 5).

Since the subjects were fasting the trend was not due to the ingestion of fluoride nor was it in our study because, as can be seen by comparing Figs. 1 and 2A, the changes in plasma [F] were not associated with the meal times.

There was a clear tendency for the average plasma [F] to decline during the 5 days of the present study (Table 1). This can be explained by the dynamic relationship between F in the extracellular fluids and the exchangeable pool of bone which has been called the blood–bone steady-state [4,10]. According to this concept, there is a relatively fixed ratio between the [F] in the exchangeable pool of bone and the surrounding extracellular fluid which is continuous with plasma. There is a net transfer of F from extracellular fluids to bone whenever plasma concentrations increase and a net transfer from bone to plasma when plasma concentrations decrease. The subjects began drinking non-fluoridated water and ceased the use of fluoridated toothpaste 30 days prior to the start of the study. This was done with the expectation that the amount of F in the exchangeable bone pool would have been largely exhausted so that there would have been little day-to-day change in the plasma concentrations during the 5-day study. The observation that there was, in fact, a decline in plasma concentrations during the study suggests that F was still being depleted from bone but at a progressively slower rate. It is not known how long F can be mobilized from the exchangeable pool but it appears to continue beyond 30 days.

Moreover, when day 1 was analyzed separately from days 2–5, the same profile during the day was observed, despite the higher plasma F for day 1 when compared to the others. This again reinforces the existence of a biological rhythm for the plasma F concentration, independently of the plasma levels, with the peak occurring between 8 and 11 a.m., and the trough occurring between 5 and 8 p.m. (Fig. 2A). The higher plasma F found for

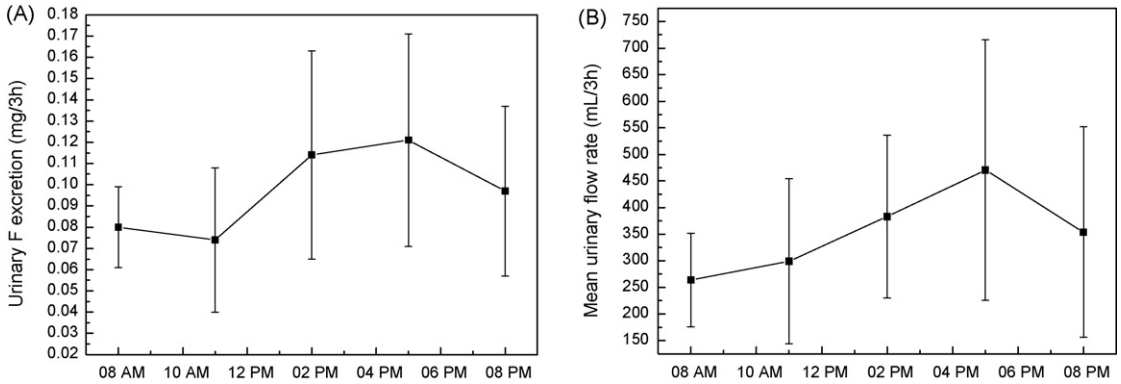

Fig. 4. (A) Urinary fluoride excretion (mg/3 h) as a function of the time of urine collection. (B) Urinary flow rate (mL/3 h) as a function of the time of urine collection. Data are shown as mean (±SD). (n = 5).

Table 2
Correlation among the parameters evaluated for all the volunteers.

Variables	r	p
Plasma [F] × serum [PTH]	0.2035	0.0252*
Plasma [F] × plasma [Ca]	−0.1046	0.2517
Plasma [F] × plasma [P]	−0.0356	0.7042
Plasma [F] × urinary F excretion	0.3296	0.0008*
Plasma [F] × urinary flow	−0.2159	0.0262*
Urinary flow × urinary F excretion	0.3873	0.0001*
Serum [PTH] × plasma [Ca]	−0.3230	0.0003*
Serum [PTH] × urinary F excretion	0.2624	0.0087*

* Statistically significant ($p < 0.05$).

the 8 a.m. on day 1 was influenced by the subjects 1, 2 and 5 (Table 1). The difference for the plasma F found for the first day when compared to the others suggests that when F intake is reduced, the higher variation on plasma F due to the exchange between bone and plasma occurs 12–24 h later. From day 1 of the present study, the subjects started to receive the standardized low-F diet.

The positive correlation between plasma [F] and serum PTH concentration confirms data published by Waterhouse et al. [11] (Table 2). PTH is one of the rapidly acting hormones. Small increases in plasma calcium concentrations have been shown even during the first hour following the administration of PTH in humans. Because most of the calcium comes from bone, it would be expected that plasma [F] would change in the same way [4–6]. The negative correlation found between serum PTH and plasma calcium concentration provides additional support for this explanation (Fig. 3A and B). That is, when plasma calcium concentrations fall, PTH release increases. This promotes an increase in plasma calcium concentrations, followed by an increase in plasma F concentrations. Despite the calcium content of the meals ingested in the present study was not measured, we cannot discharge the possibility that the increase in plasma calcium concentration seen at 11 a.m. may also have been due to the intake of milk (200 mL) during breakfast. This was the only occasion during the day when a food expected to have high calcium was ingested. If this is so, it is probable that higher plasma calcium levels at 11:00 a.m. (Fig. 3B) have determined low PTH levels at this time (Fig. 3A). The negative correlation between serum PTH and plasma calcium (Table 2) suggests that calcium is controlling PTH secretion. Since this hormone increases bone resorption, more fluoride originally incorporated in bone is released into plasma, what helps to explain the positive correlation between PTH and fluoride concentrations in plasma (Table 2).

The positive correlation between plasma [F] and urinary fluoride excretion was expected (Figs. 2A and 4A, Table 2), because the F in urine comes from plasma [4]. So, if certain variables such as glomerular filtration rate [11,12], urinary pH [13–15] and urinary flow rate [16] are relatively constant in an individual, then any increase (or decrease) in plasma [F] would be followed by an increase (or decrease) in the F excretion. This explains why the renal clearance of fluoride is not affected by changes in plasma [F] [4].

The positive correlation between plasma PTH concentration and the urinary excretion rate of F (Figs. 3A and 4A, Table 2) can also be explained in the same way, i.e., PTH increased plasma [F] which, in turn, increased urinary F excretion.

In agreement with the findings by other investigators [17–19], a positive correlation between urinary F excretion and urinary flow rate was observed (Fig. 4A and B, Table 2). However, there may not be a causal relationship between these two variables because when urinary flow rate increases it dilutes the concentration of hydrogen ions which, in turn, increases the pH within the tubular fluid. This decreases the HF concentration and, therefore, F reabsorption from the renal tubules [15].

One interesting finding was the increase in urinary flow rate after 11:00 a.m. (Fig. 4B), which was coincident with a decrease in plasma fluoride levels after this time (Fig. 2A). Considering the above-mentioned possibility that the fluctuations in PTH levels observed are mainly due to calcium intake from milk ingested in the breakfast, it can be hypothesized that the observed decrease in plasma fluoride concentrations appears to be more a consequence of the increase in the excretion of fluoride in urine than an effect of PTH.

4. Concluding remarks

The results taken together show the occurrence of daily variations in human plasma [F] and suggest that the renal system is more likely to control these fluctuations. Further studies should be conducted in order to investigate the possible existence of a circadian rhythm for human plasma [F].

5. Experimental

5.1. Volunteers

Ethical approval for the study was obtained from the Bauru Dental School, University of São Paulo Local Research Ethics Committee. Five healthy adults (V1–V5), aged between 27 and 33 years, from both genders, participated in this study. A written informed consent was obtained from all subjects.

5.2. Experimental procedure

Participants lived in Bauru, State of São Paulo, Brazil (0.6–0.8 ppm fluoride in the drinking water). They were chosen because they drank non-fluoridated mineral water and cooked with water from the public supply. Thirty days prior to the beginning of the study, the subjects refrained from using fluoridated dental products and from ingesting relatively highly fluoridated food or drink products including marine fish and tea. During the 5-day experimental period all the volunteers stayed in the same house supplied with non-fluoride water and received a standardized low-fluoride diet (<0.3 mg/day) to minimize diet-related changes in plasma [F]. Fifteen days prior to the beginning of the study the female participants refrained from using any kind of hormones. During the study, the volunteers did not practice any exercising.

Present and past history of the subjects indicated that they were healthy.

5.3. Blood collection

To determine the diurnal variation in plasma fluoride levels, blood samples were taken from the arm vein of subjects at the following times: 8 a.m., 11 a.m., 2 p.m., 5 p.m. and 8 p.m., during 5 days. Between 8 p.m. and 8 a.m. no samples were taken. The 2 p.m. blood collection was done 30 min after the subjects had had lunch. A blood volume of approximately 16 mL was collected each time (8 mL were placed in heparinized plastic tubes, containing 15 μL heparin containing 0.184 μg F/mL, while the remaining 8 mL were placed in plastic tubes with no additives).

5.4. Urine collection

Urine samples were collected every 3 h, from 8 a.m. to 8 p.m. and additionally from 8 p.m. to 8 a.m., in wide-necked plastic flasks, labeled with a colored, easily recognizable individual

sticker. Volume of each individual sample was determined, and an aliquot (50 mL) was frozen until F analysis.

Data from urine samples collected at times 8 p.m. to 8 a.m., 8 a.m. to 11 a.m., 11 a.m. to 2 p.m., 2 p.m. to 5 p.m. and 5 p.m. to 8 p.m. were correlated to data from blood samples collected at times 8 a.m., 11 a.m., 2 p.m., 5 p.m. and 8 p.m., respectively.

5.5. Duplicate diet

In order to estimate F intake from the diet, the duplicate diet method was used. A portion of the standardized diet given to the subjects was reserved. Everything that the subjects did not ingest, like bones, peels, skins, was removed. Samples were homogenized with known volumes of deionized water and frozen in labeled plastic vials until F analysis. Since the only beverage the subjects drank during the day was non-fluoridated water, the liquids ingested were not collected.

5.6. Analysis of F

Blood samples were centrifuged at 3000 rpm (Jouan A14) for 5 min for plasma separation. Plasma was analyzed in duplicate with the ion-specific electrode (Orion Research, Cambridge, MA, USA, model 9409) and a miniature calomel reference electrode (Accumet, #13-620-79), after overnight hexamethyldisiloxane (HMDS) facilitated diffusion, according to Taves' method [20], as modified by Whitford [4]. Prior to diffusion, 200 μL of F-free H_2SO_4 was added to the 1.00 mL samples and left open to the atmosphere for 15 min, in order to remove CO_2. During the diffusion process, which was conducted at room temperature, the solutions in the non-wettable Petri dishes (Falcon, No. 1007) were gently swirled on a rotatory shaker. Fluoride standards (0.25, 0.50, 1.00, 5.00 and 10.00 nmol) were prepared in triplicate and pre-diffused with F-free H_2SO_4 in the same manner as the samples by serial dilution of a stock standard containing 0.1 M fluoride (Orion 940906). Diffused standards, which differed from the pre-diffused standards only by non-addition of F-free H_2SO_4 were also prepared. The final volume of the samples and standards was adjusted to 75 μL with deionized water. Thus, considering the limit of detection of the ion-specific electrode as 0.02 μg F/mL, it was possible to analyze, with accuracy, plasma samples with a volume superior than 0.5 mL containing more than 0.0095 μg F/mL. In addition, nondiffused F standards were prepared with the same solutions (0.05 M NaOH, 0.20 M acetic acid, plus NaF) that were used to prepare the diffused standards and samples. The nondiffused standards were made up to have exactly the same F concentrations as the diffused standards. Comparison of the millivolt readings demonstrated that the F in the diffused and pre-diffused standards had been completely trapped and analyzed (recovery >99%). The millivolt potentials were converted to μg F using a standard curve with a correlation coefficient of $r \geq 0.99$.

F in urine samples was analyzed using the ion-specific electrode (Orion Research, Cambridge, MA, USA, model 9609), after sample buffering with an equal volume of TISAB (Total ionic strength adjustment buffer). A set of standards (containing 0.05, 0.10, 0.20, 0.40, 0.80 and 1.60 ppm fluoride) was prepared, using serial dilution from a 100 ppm NaF stock solution (Orion). The millivolt potentials were converted to ppm F using a standard curve with a correlation coefficient of $r \geq 0.999$.

Diet and water F analysis were carried out in duplicate after HMDS facilitated diffusion, as for the plasma, except for the addition of F-free H_2SO_4 to the samples.

5.7. Hormone analysis

Serum parathormone (PTH) was analyzed by chemiluminescence.

5.8. Calcium and phosphate analysis

Plasma calcium and phosphate were analyzed by AAS and colorimetry [21], respectively.

5.9. Statistical analysis

The assumptions of equality of variances and normal distribution of errors were checked, using Bartlett and Kolmogorov–Smirnov tests, respectively. Data were then analyzed for statistically significant differences by repeated-measures ANOVA and Tukey's post hoc test and Pearson's correlation coefficient ($p < 0.05$).

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References

[1] W.S. Guy, D.R. Taves, in: Proceedings of the International Association for Dental Research, 1973, Abstract 718.
[2] J. Ekstrand, Caries Res. 12 (1978) 123–127.
[3] D.C. Cowell, W.H. Taylor, Ann. Clin. Biochem. 18 (1981) 76–83.
[4] G.M. Whitford, The Metabolism and Toxicity of Fluoride, Karger, Basel, 1996, pp. 156.
[5] A.M. Perault-Staub, J.F. Staub, G. Milhaud, Endocrinology 95 (1974) 480–487.
[6] R.V. Talmadge, J.H. Roycroft, G. Milhaud, Calcif. Tissue Res. 17 (1975) 19–26.
[7] J.C. Leloup, A. Goldbeter, Bioessays 30 (2008) 590–600.
[8] N. Mrosovsky, P.A. Salmon, Chronobiol. Int. 11 (1990) 343–348.
[9] H. Husdan, R. Vogl, D. Oreopoulos, C. Gryfe, A. Papoport, Clin. Chem. 22 (1976) 1884–1888.
[10] W.F. Neuman, M.W. Neuman, The Chemical Dynamics of Bone Mineral, University of Chicago Press, Chicago, 1958, pp. 75–100.
[11] C. Waterhouse, D.R. Taves, A. Munzer, Clin. Sci. 58 (1980) 145–152.
[12] H.H. Schiffel, U. Binswanger, Nephron 26 (1980) 69–72.
[13] J. Ekstrand, M. Ehrnebo, G.M. Whitford, P. Jarnberg, Eur. J. Clin. Pharmac. 18 (1980) 189–194.
[14] J. Ekstrand, C.J. Spak, M. Ehrnebo, Acta Pharmacol. Toxicol. 50 (1982) 321–325.
[15] G.M. Whitford, J. Dent. Res. 69 (1990) 539–549, Special issue.
[16] P.O. Jarnberg, J. Ekstrand, M. Ehrnebo, Toxicol. Lett. 18 (1993) 141–166.
[17] H. Schiffel, U. Hofmann, M. Huggler, U. Binswanger, Nephron 29 (1981) 245–249.
[18] J. Ekstrand, S.J. Fomon, E.E. Ziegler, S.E. Nelson, Pediatr. Res. 35 (1994) 157–163.
[19] R.J. Baez, M.X. Baez, T.M. Marthaler, Rev. Panam. Salud. Publica. 7 (2000) 242–248.
[20] D.R. Taves, Talanta 15 (1968) 969–974.
[21] P.S. Chen Jr., T.Y. Toribara, H. Warner, Anal. Chem. 28 (1956) 1756–1758.