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The Effect of Age and Gender on Calcium, Phosphorus, and Calcium-Phosphorus Ratio in the Crowns of Permanent Teeth

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Abstract

Objectives: The aim of the current study was to evaluate quantitatively the effect of age and gender on calcium, phosphorus, and calcium-phosphorus ratio in the crowns of permanent teeth.

Methods: Calcium (Ca) and phosphorus (P) mass fractions as well as Ca/P mass fraction ratio were estimated in intact tooth crown samples from apparently healthy humans, 38 women and 46 men, aged from 16 to 58 years. For Ca and P mass fractions measurements, instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides was used.

Results: Mean values (mean ± standard error of mean, on dry mass basis) for the investigated parameters in two age groups (16-35 and 36-55 years) of females were: Ca 349 ± 11 g/kg, P 185 ± 8 g/kg, Ca/P ratio 1.94 ± 0.06 and Ca 353 ± 12 g/kg, P 186 ± 8 g/kg, Ca/P ratio 1.92 ± 0.07, respectively. The investigated parameters in the same age groups of males were: Ca 357 ± 9 g/kg, P 188 ± 9 g/kg, Ca/P ratio 1.96 ± 0.09 and Ca 341 ± 5 g/kg, P 169 ± 3 g/kg, Ca/P ratio 2.03 ± 0.04, respectively. The mean values for Ca, P and Ca/P ratio in the tooth crown were within a very wide range of reference data for tooth enamel and close to their median.

Conclusions: A tendency for reduced Ca and P mass fraction and changed Ca/P ratio in crown of female and male teeth after 35 years of age as well as differences between these parameters for female and male in age range 16-58 years was not observed.

Keywords: *Human tooth crown; calcium; phosphorus; Ca/P ratio; neutron activation analysis*

Abbreviations

INAA-SLR: Instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides

Introduction

Enamel that covers the anatomical crown of a tooth is the hardest mineralized tissue in the human body. Enamel is one of the most important structures of the tooth, both from a functional and esthetic point of view. Dental enamel develops under direct physiological control of the organism and reflects past metabolic, nutritional, and pollution events [1]. Mature enamel contains no active cells and consists approximately 96% inorganic bioapatite crystal, mostly hydroxyapatite ((Ca10(PO4)6(OH)2) crystals, 1-2% organic matter and 2-3% water [2]. Calcium (Ca) and phosphorus (P) are the main chemical elements of hydroxylapatite. The mass fraction of Ca in pure hydroxyapatite is 39.9%, the mass fraction P - 18.5%, and stoichiometric Ca/P mass fraction ratio - 2.15. It was well documented that a hardness of human tooth enamel depends on the Ca and P contents as well as Ca/P ratio [3]. Thus, Ca and P contents play an important role in enamel condition and health. Deficiency and excess of these chemical elements, resulting from metabolic disorders as well as exposure to both the natural and man-made environment, can lead to a wide variety of clinical effects such as enamel hypoplasia, susceptibility to caries and others.

Once enamel is completely formed, it does not have the ability for further growth or repair, but it does have the ability to remineralize. This means that areas experiencing early demineralization are able to regain minerals from the enamel environment. The enamel environment starts at the enamel surface. Enamel crystals are in physico-chemical equilibrium with the fluids that bathe them. Thus, a tidal effect constantly occurs, whereby the enamel crystallites are dissolved and then recrystallized [4]. This process of demineralization and remineralization can occur without loss of enamel structure when adhering to proper nutrition and oral care. Since enamel is a biomaterial that is structurally adapted to different chewing functions and loading situations in tooth, the exact composition may vary depending on gender, age, type of tooth and its site, but also with alterations known to occur in dental diseases. Thus, such characteristics of enamel as the reference normal levels of Ca and P content as well as Ca/P ratio subject to age and gender are important in diagnostics of dental diseases and control of tooth treatment including bleaching, laser drilling and so on.

Teeth and bones have many similar physical and chemical properties [5]. As was shown by us in previous studies [6-20] the Ca and P mass fractions in different bones depend from age and gender. These findings allow us assume also the age- and gender dependence of Ca and P mass fractions in permanent teeth.

The tooth crown was chosen for our investigation because it consisting mainly of enamel. If use the tooth crown as the subject of study, there is no necessity to separate any tooth tissue. To our knowledge, only one report is available on Ca and P mass fraction in permanent teeth crown of children [21]. No data are available for the Ca and P mass fractions and also Ca/P ratio in teeth crowns of adults.

Enamel comprises the main portion of the tooth crown. There are many studies regarding Ca and P determination in tooth enamel, using chemical techniques and instrumental methods [22-73]. However, the majority of these data are based on measurements of processed tissue. First of all, enamel samples can be chemically contaminated in the course of sampling [74,75]. In many studies enamel samples are ashed before analysis. In other cases, enamel samples are treated with solvents (distilled water, ethanol, formaldehyde etc) and then are dried at high temperature for many hours. There is evidence that certain quantities of chemical elements, including Ca and P, are lost as a result of such treatment [75-77]. Moreover, only few of these studies employed quality control using certified reference materials (CRM) for determination of the chemical element mass fractions.

In our previous reports it was shown that instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) is an adequate analytical tool for the non-destructive precise determination of Ca and P mass fraction in intact teeth roots [78].

The primary purpose of this study was to determine reference values for the Ca and P mass fraction and Ca/P mass fraction ratio in the intact crown of permanent teeth using INAA-SLR. The second aim was to evaluate the quality of obtained results. The third aim was to compare the mean values of Ca and P mass fraction and Ca/P mass fraction ratio in different age groups in the period of life from 16 to 58 years. The third aim was to estimate the difference between Ca and P mass fraction and Ca/P mass fraction ratio in the teeth crowns of males and females The final aim was to compare the results obtained in the study with published data for the tooth enamel.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

Materials and Methods

Samples

Non carious permanent teeth were collected at the Department of Forensic Medicine of the Obninsk Hospital. The molars and premolars were extracted early after death at necropsy (within 24 hours) from 38 women (age range 16-55 years) and 46 men (age range 16-58 years). One tooth was obtained from each subject. The typical causes of death in most of these subjects included traffic accident, occupational injury and domestic trauma. All the deceased were citizens of Obninsk. None of those who died a sudden death had suffered from any systematic or chronic disorders before.

Sample preparation

After extraction teeth were immediately frozen at - 18°C until use. A titanium tool was used to cut and to scrub soft tissue and blood off the crowns. After separating the crowns from roots with a titanium knife, samples were freeze dried until constant mass was obtained. Only the crowns were used in this study. After drying crowns were weighed and sealed in thin polyethylene films washed with acetone and rectified alcohol beforehand. The sealed samples were then placed in labeled polyethylene ampoules.

Method and reference materials

A horizontal channel in the pneumatic rabbit system of the WWR-c research nuclear reactor was used to determine Ca and P mass fractions by INAA-SLR. Ten subsamples of the standard reference material NIST SRM1486 bone meal and certified reference material IAEA H-5 animal bone was analyzed under the same conditions as teeth crowns samples to estimate the precision and accuracy of results.

The basement of INAA is the irradiation of stable atoms in the sample by neutrons, the transmutation of atoms in radionuclides, and the spectrometry of their self-radiations. Details of nuclear reactions, radionuclides, gamma-energies, methods of analysis and the results of quality control were presented in our earlier publications concerning the chemical elements of human bones [6-14,16,17].

Computer programs and statistic

A dedicated computer program of INAA-SLR mode optimization was used [79]. Using the Microsoft Office Excel software to provide a summary of statistical results, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for Ca and P mass fractions and Ca/P mass fraction ratio obtained. The difference in the results between all age groups and between males and females was evaluated by Student's parametric t-test. For the construction of "age-Ca/P ratio" diagram and the estimation of the Pearson correlation coefficient between the Ca and P mass fractions the Microsoft Office Excel software was also used.

Results

Table 1 shows INAA-SLR results for Ca and P mass fractions (g/kg, dry mass basis) in ten sub-samples of the standard reference material NIST SRM1486 bone meal and certified reference material IAEA H-5 animal bone compared to certified values.

Element	CRM IAEA H-5 certified values		This work results (n = 10)	SRM NIST 1486 certified values		This work results (n = 10)
	Mean	Type	Mean ± SD	Mean	Type	Mean ± SD
Ca	212	C	208 ± 2	266	C	271 ± 8
P	102	C	94.3 ± 0.8	123	C	119 ± 3

Table 1: *Instrumental neutron activation analysis data of Ca and P mass fractions (g/kg, dry mass basis) in the CRM IAEA H-5 Animal Bone and SRM NIST 1486 Bone Meal reference materials compared to certified values.*

Mean: Arithmetic mean; SD: Standard deviation; C: Certified values

Table 2 gives some statistical parameters of the Ca and P mass fractions and Ca/P mass fraction ratio such as arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels in the intact crowns of sound permanent teeth of females and males separately, as well as females and males combined.

Tissue	Parameter	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Female	Age, years	36.6	11.8	1.9	16	55	37.5	16.9	55.0
n = 38	Ca	352	48.1	8.4	234	486	360	262	429
	P	186	33.4	5.7	112	258	185	132	255
	Ca/P ratio	1.94	0.27	0.05	1.40	2.65	1.92	1.42	2.48
Male	Age, years	36.5	11.4	1.7	16	58	37.5	17.1	55.0
n = 46	Ca	348	32.0	4.9	296	436	344	307	434
	P	177	29.8	4.6	110	248	176	118	247
	Ca/P ratio	2.00	0.28	0.04	1.40	2.96	2.01	1.67	2.79
Female	Age, years	36.5	11.5	1.3	16	58	37.5	17.0	55.0
and male	Ca	350	39.6	4.6	234	486	347	279	434
(combined)	P	181	31.5	3.6	110	258	180	117	249
n = 84	Ca/P ratio	1.97	0.28	0.03	1.40	2.96	1.93	1.42	2.67

Table 2: *Some statistical parameters of Ca and P mass fractions (g/kg, dry mass basis) and Ca/P mass fraction ratio in the crowns of sound permanent teeth of females and males.*

M *- arithmetic mean;* ***SD*** *- standard deviation;* ***SEM*** *- standard error of mean;* ***Min*** *- minimum value;* ***Max*** *- maximum value;* ***P 0.025*** *- percentile with 0.025 level;* ***P 0.975*** *- percentile with 0.975 level.*

Table 3 depicts our data for mean values (mean ± standard error of mean) of Ca and P mass fractions and Ca/P mass fraction ratio in the teeth crowns of healthy women and men from 16 to 58 years in 8 subgroups of 5 years (8th subgroup of male tooth crown from 51 to 58 years). Mean values (mean ± standard error of mean) for these subgroups (SG) were: SG1 - 19.0 ± 1.0, SG2 - 23.3 ± 0.8, SG3 - 29.2 ± 0.6, SG4 - 33.6 ± 0.6, SG5 - 38.2 ± 0.4, SG6 - 42.1 ± 0.6, SG7 - 47.8 ± 0.5, SG8 - 54.7 ± 0.8 years. Age-dependence of the Ca/P mass fraction ratio in crown of sound permanent teeth of males for period of life 16-58 years was found using the means of age and Ca/P mass fraction ratio in 8 subgroups of crown samples (Figure 1).

Sub-group	Age range years	Female				Male			
		n	Ca	P	Ca/P	n	Ca	P	Ca/P
SG1	16-20	5	324 ± 22	173 ± 11	1.91 ± 0.09	5	326 ± 19	148 ± 38	2.32 ± 0.47
SG2	21-25	4	319 ± 21	157 ± 21	2.08 ± 0.14	5	361 ± 20	184 ± 22	2.06 ± 0.23
SG3	26-30	4	368 ± 4	209 ± 13	1.95 ± 0.11	5	370 ± 15	200 ± 11	1.85 ± 0.06
SG4	31-35	5	380 ± 12	200 ± 10	1.90 ± 0.04	7	355 ± 19	195 ± 15	1.85 ± 0.11
SG5	36-40	4	359 ± 14	186 ± 11	1.95 ± 0.10	5	346 ± 10	180 ± 3	1.92 ± 0.06
SG6	41-45	5	318 ± 13*	172 ± 14	1.90 ± 0.17	8	334 ± 9	166 ± 5*	2.02 ± 0.06
SG7	46-50	6	335 ± 24	168 ± 10*	2.03 ± 0.18	5	341 ± 4	166 ± 10	2.07 ± 0.11
SG8	51-58	5	399 ± 24	223 ± 16	1.80 ± 0.09	6	348 ± 11	167 ± 8	2.09 ± 0.05*

Table 3: *Mean values (MSEM) of Ca and P mass fractions and Ca/P mass fraction ratio in the crowns of sound permanent teeth depending on age (g/kg, dry mass basis).*

*M: Arithmetic mean, SEM: Standard error of mean, * p < 0.05 Student's t-test (compared to data for age group 26-30 years of females and males, respectively).*

In order to estimate the effect of age on the investigated parameters we used also two combined groups: one with young people, 16-35 years, and one with older people, 36-58 years, because in our previous study was found that the Ca and P mass fractions in different bones begin to decrease after 35 years [15-20]. For female and male, results are shown in Table 4.

Parameter	Female			Male		
	16-35 year n = 18	36-55 year n = 20	*P* Student's *t*-test	16-35 year n = 22	36-58 year n = 24	*P* Student's *t*-test
Ca	349 ± 11	353 ± 12	N.S.	357 ± 9	341 ± 5	N.S.
P	185 ± 8	186 ± 8	N.S.	188 ± 9	169 ± 3	N.S.
Ca/P	1.94 ± 0.06	1.92 ± 0.07	N.S.	1.96 ± 0.09	2.03 ± 0.04	N.S.

Table 4: *Effect of age on mean values (M ± SEM) of Ca and P mass fractions (g/kg, dry mass basis) and Ca/P mass fraction ratio in the crowns of sound permanent teeth.*

M: Arithmetic mean; SEM: Standard error of mean; N.S.: Not Significant

More or less steady age distributions of female and male data within the range 15-58 years allows us to use all data for female and male separately to detect if there are any differences related to gender. For this purpose, three age groups 16-35, 36-58, and 16-58 years were used (Table 5). In the right column of Table 5 there is additional information concerning the same parameters for female and male together in each age group.

Age group years	Parameter	Gender		*p* Student's *t*-test	Females and males (combined)
		Females	Males		
16 - 35	Ca	349 ± 11	357 ± 9	N.S.	354 ± 7
	P	185 ± 8	188 ± 9	N.S.	187 ± 6
	Ca/P	1.94 ± 0.06	1.96 ± 0.09	N.S.	1.95 ± 0.05
36 - 58	Ca	353 ± 12	341 ± 5	N.S.	347 ± 6
	P	186 ± 8	169 ± 3	N.S.	177 ± 4
	Ca/P	1.92 ± 0.07	2.03 ± 0.04	N.S.	1.98 ± 0.04
16 - 58	Ca	352 ± 8	348 ± 5	N.S.	350 ± 5
	P	186 ± 6	177 ± 5	N.S.	181 ± 4
	Ca/P	1.94 ± 0.05	2.00 ± 0.04	N.S.	1.97 ± 0.03

Table 5: *Effect of gender on mean values (M ± SEM) of Ca and P mass fractions (g/kg, dry mass basis) and Ca/P mass fraction ratio in the crowns of sound permanent teeth.*

M - arithmetic mean; SEM - standard error of mean; N.S - not significant

Comparison of reference data for tooth enamel with our results for Ca and P mass fractions as well as for Ca/P mass fraction ratio in tooth crown is shown in Tables 6 to 8, respectively. All reference data for Ca and P are given in Tables 5 and 6 on dry mass basis. Some values for Ca and P mass fraction in Table 6 and 7 were not given by the authors on dry mass basis but were calculated by us using the median values of water and ash content in the tooth enamel, 3% and 96% on wet mass basis, respectively, taken from reference data [2,42,80]. Some values for Ca/P ratio shown in Table 8 were also calculated by us using the mean of Ca and P mass fraction reported by the same authors (Table 6 and 7).

Discussion

The means for Ca and P mass fractions in ten sub-samples of the IAEA H-5 animal bone and NIST SRM1486 bone meal reference materials determined by INAA-SLR were in a good agreement with mean values of the certificates (Table 1). Good agreement with the certified data of CRM indicated an acceptable accuracy of the results obtained in the study of Ca and P mass fractions in teeth crowns presented in Tables 2-5.

The mean values for Ca and P mass fractions as well as for Ca/P mass fraction ratio, as shown in Table 2, agreed well with medians. It indicated that the distribution of individual data of investigated parameters was close to a normal law and confirmed an appropriateness using the Student's parametric t-test in the study.

The Ca mass fraction in tooth crown of female increased in the second to fourth decades and it was maintained in the nearly stable level at the age above 30 years (Table 3). In the period of life from 16 to 25 years the Ca mass fraction was 1.25 times lower than in teeth crowns of females aged 51 to 55 years. The same tendency of age-dependency was found for P mass fraction in female teeth crowns. The Ca and P mass fraction in tooth crown of male reached a maximum at the age of 30 years, i.e. at the same age that in tooth crown of female (Table 3). However, in contrast to female the Ca and P mass fraction in tooth crown of male decreased at the age above 30 years. The means of Ca/P mass fraction ratio were maintained in the range 1.80-2.08 in female teeth crowns and did not change with age. The means of Ca/P mass fraction ratio in male teeth crowns decreased in the second to fourth decades, reached a minimum at the age of 30-35 years, and above 35 years began to increase (Figure 1). The mean of Ca/P mass fraction ratio at the age 51-55 years was significantly higher (+13%) than the minimal value of this ratio at the age about 30 years (Table 3).

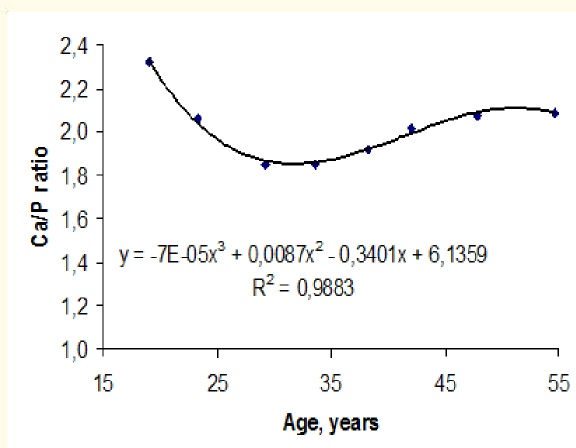

Figure 1: *Age-dependence of the Ca/P mass fraction ratio in crown of male permanent teeth.*

A tendency for reduced Ca and P mass fraction by age was not observed in the comparison between two combined age groups (15-35 and 36-58 years) of females and males (Table 4). Significant differences on the investigated parameters related to gender were also not found in these two age groups (Table 5).

Reference	Year of publication	Method	n	Age years	Treatment of samples	Ca mass fraction	
						M, M ± SD, range of M	Range*
[22]	1937	Chem	42	Adult	PF, D, G, F, AD	354 ± 10	-
[23]	1950	Chem	-	Adult	-	387	-
[24]	1962	Chem	240	Adult	-	346 - 371	-
[25]	1967	INAA	-	Adult	-	374 ± 10	-
		Chem	-	Adult	PF, Ash, AD	-	336-394
[26]	1967	Chem	-	Adult	Ash, AD	360 - 375	-
[27]	1967	PIGE	-	Adult	F, P, D	-	278-381
[28]	1969	EPMA	-	Adult	Em, P	-	371-387
[29]	1971	INAA	8	Adult	D, G	360 ± 6	-
[30]	1974	INAA	175	10-90	F, D	362 - 377	-
[31]	1975	INAA	-	Adult	D, G	370 ± 6	-
[32]	1976	PIXE	1	Adult	P	-	309-361
[33]	1977	INAA	20	Adult	D, G	379 - 380	-
[34]	1981	RD	80	15-25	FF, Em, P	-	367-409
[35]	1981	PIXE	30	Adult	P	371 ± 33	-
[36]	1981	PIXE	1♂	65	Em, P	373	-
[37]	1982	RNAA	1	Adult	-	379	-
[38]	1982	AAS	22	Adult	Ash, AD	361	-
[39]	1984	EPMA	39	24-45	-	386 ± 27	-
[40]	1985	RNAA	-	Adult	-	309 ± 31	-
[41]	1987	Chem	7	Adult	Ash, AD	304 ± 68	-
[42]	1988	-	-	Adult	-	372	-
[43]	1989	Chem	16	14	AD	418 ± 25	-
[44]	1990	EPMA	34	Adult	P	126 ± 17	-
[45]	1990	PIXE	6	Adult	-	368	
[46]	1991	AES	30	31-50	Ash	278 ± 1	-
[21]	1991	PIXE	13	> 10	F, PF, G	(340 ± 98)	-
[47]	1991	MS	6	67-96	F, D, FF, AD	-	363-386
[48]	1997	EPMA	-	Adult	Em, P	-	173-292
[49]	1997	PIXE	86	Adult	D	171 - 279	-
[50]	2003	EPMA	3	Adult	-	175 - 182	-
[51]	2005	XRF	32	Adult	FF	439 ± 82	-
		INAA	32	Adult	FF	546	-
[52]	2005	PIXE	10	Adult	P	~210	-
[53]	2005	-	-	Adult	-	570	-
[54]	2006	EPMA	7	Adult	FF, PF	376 ± 5	-
[55]	2008	INAA	10	Adult	F	321 ± 49	233-424
[56]	2009	INAA	9	Adult	F	332 ± 43	

[57]	2010	EPMA	8	Adult	F, B, P	376	-
[58]	2010	PIXE	5	Adult	AC, F	-	350-447
[59]	2011	EPMA	5	Adult	F, P	284 ± 83	-
[60]	2011	INAA	30	Adult	F, D, G	361 ± 10	-
		ICP-AES			F, D, G, AD	361 ± 10	-
[61]	2013	ICP-MS	80	Adult	-	288 ± 41	-
[62]	2014	EPMA	16	>10	P, F	26-270	-
[63]	2015	EPMA	10	Adult	-	297	-
This work		INAA		16-58	Intact	350 ± 40	234-486

All references (n = 39)	Median of means	Range of means
	361	126-570

Table 6: *Reference data of Ca mass fraction (g/kg, dry mass basis) in enamel (crown) of sound permanent teeth.*

**Range of individual results, "-"- no information; Mean - arithmetic mean; SD - standard deviation; Chem - chemical method; INAA - instrumental neutron activation analysis; PIGE - proton induced gamma-ray emission; EPMA - electron probe microanalysis; PIXE - proton induced X-ray emission; RD - dispersion of double refraction; AAS - atomic absorption spectrophotometry; RNAA - radiochemical neutron activation analysis; AES - atomic emission spectroscopy; MS- mass spectrometry; XRF - X-ray fluorescence analysis; ICP-AES - inductively coupled plasma atomic emission spectrometry; ICP-MS - inductively coupled plasma mass spectrometry; PF -protein, fat free; D - drying at high temperature; G - grinding; F - flotation (washing); AD - acid digestion; Ash - ashing; P - polishing; Em - embedding in balsam or plastic; FF - fixation by formalin or alcohol; B - kept in buffered saline; AC - acid cleaned*

For almost lack of reported data of chemical element contents in permanent tooth crown we compared our results with published data on tooth enamel. It was acceptable because a tooth crown mainly consists of enamel. As it was shown in Table 6 there was a coincidence between our mean value of Ca mass fraction in female and male tooth crown combined, 350 ± 40 (M ± SD) g/kg on dry mass basis, and the median reference value, 361 g/kg on dry mass basis. The obtained mean of Ca mass fraction in tooth crown agreed well with the only result 340 ± 98 (g/kg on dry mass basis) reported for permanent tooth crown of children [21]. Our mean value for P mass fraction, 181 ± 4 (M ± SD) g/kg on dry mass basis, was also close to the median reference one, 176 g/kg on dry mass basis (Table 7). Standard deviations found for Ca and P mass fractions in crown were respectively large but laid in the same level as some published data for enamel (Tables 6 and 7). This was the consequence of the very wide individual variation in Ca and P mass fractions in intact crown (Tables 6 and 7).

The median reference value for Ca/P mass fraction ratio in enamel, 2.02 (Table 8), was lower than the stoichiometric value for hydroxyapatite, 2.15, but very close to ours for crowns, 1.97 ± 0.28 (M±SD). The differences were probably due to the presence of other Ca-phosphates in enamel differ from hydroxyapatite, which increased the P mass fraction and decreased the Ca/P mass fraction ratio in enamel, respectively. If the crown consisted of pure hydroxyapatite the correlation between Ca and P mass fractions should be ideal ($r = 1.0$). The presence of other Ca-phosphates in enamel in different proportions reduced the strong correlation between Ca and P mass fractions. This correlation for males and females aged 16 to 35 years was $r = 0.71$ ($p < 0.0001$) and $r=0.89$ ($p < 0.0001$), respectively. By aging the correlation becomes lower. So, for males and females at age over 36, the respective values were $r = 0.50$ ($p < 0.01$) and $r = 0.57$ ($p < 0.001$).

Tables 6-8 present a very wide range for Ca and P mass fractions and Ca/P mass fraction ratio. These values cannot be explained only on the fact that various investigators have used enamel from the sound permanent teeth of different type and from different part of teeth crowns. We consider that other reasons account for this. The majority of data shown in these Tables were based upon techniques in which the enamel sample was subjected to various treatments in order to remove organic components by washing in different solvents, or depleting the whole organic matrix by dry ashing. These treatments lead to miscalculations of the inorganic component of enamel. For example, according to Pellegrino and Biltz [81] when organic components were removed from bone by chloroform-ethanol mixtures, Ca was removed too. Other evidence [75,76,82-84] shows that upon dry ashing a great number of minerals and other biological materials are lost.

Information from other sources referring to the impact of age on the Ca and P mass fraction in enamel is very limited and contradictory. The evidence presented by Lane., *et al*. [49] demonstrated the inverse correlation for Age-Ca in enamel of permanent teeth extracted from the female donors from Oxford shire and Cornwall in the United Kingdom ($r = -0.52$, $p = 0.001$). In contrast to this result no changes in Ca and P content as well as in Ca/P ratio in enamel of permanent teeth up to the age of 90 were reported by Little., *et al*. [24], Derise., *et al*. [30], and Liu., *et al*. [61]. The U-shape line found in this study for the age-dependence of Ca/P ratio in crown of male teeth (Figure 1) was close to data for tooth enamel shown by Barhatov., *et al*. [71], but the minimal value of Ca/P ratio for enamel in the cited study was at the age of 14.

Reference	Year of publication	Method	n	Age years	Treatment of samples	P mass fraction	
						M, M ± SD, range of M	Range*
[22]	1937	Chem	42	Adult	PF, D, G, F, AD	175 ± 5	-
[23]	1950	Chem	-	-	-	181	-
[64]	1961	RNAA	15	14-16	F, G, D	183 ± 22	-
[24]	1961	Chem	240	Adult	-	175-185	-
[25]	1967	INAA	-	Adult	-	183 ± 22	-
		Chem	-	Adult	-	-	161-180
[26]	1967	Chem	146	-	Ash, AD	190-194	-
[27]	1967	PIGE	-	Adult	F, P, D	-	134-175
[28]	1969	EPMA	-	Adult	Em, P	-	175-180
[30]	1974	Chem	175	10-90	F, D, G, Ash, AD	177-192	-
[32]	1976	PIXE	1	Adult	P	-	227-258
[65]	1978	Chem	18	7-18	-	176 ± 47	-
[34]	1981	RD	80	15-25	FF, Em, P	-	168-192
[35]	1981	PIXE	30	Adult	P	166 ± 16	-
[36]	1981	PIXE	1♂	65	FF, Em, P	174	-
[37]	1982	RNAA	1	Adult	-	166	-
[39]	1984	EPMA	39	Adult	-	186 ± 22	-
[66]	1985	EPMA	9	9-10	-	188 ± 9	-
[40]	1985	RNAA	-	Adult	-	175 ± 52	-
[41]	1987	Chem	7	Adult	Ash, AD	164 ± 36	-
[42]	1988	-	-	-	-	178	-
[43]	1989	Chem	16	14	AD	175 ± 10	-
[44]	1990	EPMA	34	Adult	FF, Em, P	73 ± 17	-
[45]	1990	PIXE	6	Adult	-	176	-
[46]	1991	AES	30	31-50	Ash	180 ± 1	-
[21]	1991	PIXE	11	> 10	F, PF, G	(122 ± 44)	-
[48]	1997	EPMA	-	Adult	FF, Em, P	108-112	96-144

[50]	2003	EPMA	3	Adult	-	108-112	-
[51]	2005	XRF	32	Adult	FF	196	
		INAA	32	Adult	FF	206	
[52]	2005	PIXE	10	Adult	P	~ 95	-
[53]	2005	-	-	Adult	-	284	-
[54]	2006	EPMA	7	Adult	FF, PF	183 ± 6	-
[57]	2010	EPMA	8	Adult	F, B, P	176	-
[58]	2010	PIXE	5	Adult	AC, F	-	211-258
[59]	2011	EPMA	5	Adult	F, P	156 ± 53	-
[60]	2011	INAA	30	Adult	F, D, G	187 ± 5	-
		ICPAES			F, D, G, AD	180 ± 5	-
[61]	2013	ICP-MS	80	Adult	AD	150 ± 23	-
[62]	2014	EPMA	16	> 10	P, F	130-131	-
[63]	2015	EPMA	10	Adult	-	156	-
This work		INAA	84	16-58	Intact	181 ± 32	110-258

All references (n = 39)	Median of means	Range of means
	176	73-284

Table 7: *Reference data of P mass fraction (g/kg, dry mass basis) in enamel (crown) of sound permanent teeth.*

**Range of individual results, "-"- no information; Mean -arithmetic mean; SD - standard deviation; Chem - chemical method; RNAA - radiochemical neutron activation analysis; INAA - instrumental neutron activation analysis; PIGE - proton induced gamma-ray emission; EPMA - electron probe microanalysis; PIXE - proton induced X-ray emission; RD - dispersion of double refraction; AES - atomic emission spectroscopy; XRF - X-ray fluorescence analysis; ICP-AES - inductively coupled plasma atomic emission spectrometry; ICP-MS - inductively coupled plasma mass spectrometry; PF -protein, fat free; D - drying at high temperature; G -grinding; F - flotation (washing); AD - acid digestion; Ash - ashing; P - polishing; Em - embedding in balsam or plastic; FF - fixation by formalin or alcohol; B - kept in buffered saline; AC - acid cleaned*

Reference	Year of publication	Method	n	Age years	Treatment of samples	Ca/P mass fraction ratio	
						M, M ± SD, range of M	Range*
[22]	1937	Chem	42	Adult	PF, D, G, F, AD	2.03	-
[23]	1950	Chem	-	Adult	-	2.13	-
[24]	1962	Chem	240	Adult	-	1.96-2.10	-
[25]	1967	INAA	-	Adult	-	2.04	-
		Chem	-	Adult	-	-	1.97-2.17
[26]	1967	Chem	146	-	Ash, AD	1.86-1.94	-
[28]	1969	EPMA	-	Adult	Em, P	-	1.89-2.28
[67]	1976	ESCA	-	Adult	F, D	1.40	-
[68]	1976	AAS	87	12-14	F, D, AD	2.13-2.19	-
[69]	1976	AAS	278	12-14	F, D, AD	2.14±0.23	-
[70]	1976	EPMA	2	Adult	FF, Em, P	-	2.0-2.1

[35]	1981	PIXE	30	Adult	P	2.23±0.05	-
[71]	1981	EPMA	-	8	-	1.75	-
				14		1.55	-
				60		2.01	-
				83		2.09	-
[39]	1984	EPMA	39	Adult	-	1.61±0.13	-
[40]	1985	RNAA	-	Adult	-	1.77	-
[41]	1987	Chem	7	Adult	Ash, AD	1.85	-
[42]	1988	-	-	Adult	-	2.09	-
[43]	1989	Chem	16	14	AD	1.86±0.20	-
[44]	1990	EPMA	34	Adult	P	1.73	-
[45]	1990	PIXE	6	Adult	-	2.09	-
[46]	1991	AES	30	31-50	Ash	1.54	-
[48]	1997	EPMA	-	Adult	F, 3, Ш	-	1.69-2.02
[72]	2001	SIMS	16	Adult	F, D	1.8	1.7-1.9
[50]	2003	EPMA	3	Adult	-	-	1.62-1.63
[51]	2005	XRF	32	Adult	FF	2.20	-
		INAA	32	Adult	FF	2.78	-
[52]	2005	PIXE	10	Adult	P	2.17	-
[53]	2005	-	-	Adult	-	2.07	-
[54]	2006	EPMA	7	Adult	FF, PF	2.05±0.05	-
[57]	2010	EPMA	8	Adult	F, B, P	2.13	-
[58]	2010	PIXE	5	Adult	AC, F	-	1.60 - 1.81
[59]	2011	EPMA	5	Adult	F, P	1.82	-
[60]	2011	INAA	30	Adult	F, D, G	2.01	-
[61]	2013	ICP-MS	80	Adult	AD	1.85	-
[62]	2014	EPMA	16	> 10	P, F	2.0-2.6	-
[73]	2016	XRF	50	Adult	S, B, CUB, P	2.03±0.11	-
This work		INAA	84	16-58	Intact	1.97±0.28	1.40-2.96

All references (n = 34)	Median of means	Range of means
	2.02	1.40-2.78

Table 8: *Reference data of Ca/P mass fraction ratio in enamel (crown) of sound permanent teeth.*

**Range of individual results, "-"- no information; Mean - arithmetic mean; SD - standard deviation; Chem - chemical method; INAA - instrumental neutron activation analysis; EPMA - electron probe microanalysis; ESCA - X-ray photoelectron spectroscopy, AAS - atomic absorption spectroscopy; PIXE - proton induced X-ray emission; RNAA - radiochemical neutron activation analysis; AES - atomic emission spectroscopy; SIMS - secondary ion mass spectrometry; XRF - X-ray fluorescence analysis; ICP-MS - inductively coupled plasma mass spectrometry; PF - protein, fat free; D - drying at high temperature; G - grinding; F - flotation (washing); AD - acid digestion; Ash. - ashing; P - polishing; Em - embedding in balsam or plastic; FF - fixation by formalin or alcohol; B - kept in buffered saline; AC - acid cleaned*

The influence of gender on the Ca and P mass fraction as well as on Ca/P ratio in enamel has also received little attention, nevertheless, the results of few studies have not found significant gender-related differences in enamel for Ca and P mass fractions [30,49,64].

Conclusion

INAA-SLR has been demonstrated to be an adequate analytical tool for the non-destructive determination of Ca and P mass fractions in the intact crown of human teeth. The mean values (M ± SD) of Ca and P mass fractions (g/kg, dry mass basis) as well as Ca/P mass fractions ratio in intact crown of apparently healthy 16-58 years old women and men were: 350 ± 5, 181 ± 4, and 1.97 ± 0.03 respectively. A difference between Ca and P mass fraction as well as between Ca/P ratio in crown of female and male teeth of two age groups (up to 35 years and after 35 years) was not found. A tendency for increase Ca/P ratio in crown of male teeth after 30 years was observed. The mean values for Ca, P and Ca/P ratio in tooth crown were within a very wide range of reference data for enamel and close to their median.

Data obtained in our study expands the knowledge of physiology of dental tissues and may be used for diagnostic, therapeutic and preventive purposes. Moreover, elemental analysis, including Ca content, of human teeth is often used in paleoanthropology for dietary and environment reconstruction to assess the social and economic status of human groups [85,86] and also in forensic anthropology to determine if questionable fragments are osseous, dental, or non-skeletal in nature [87]. It is therefore evident that for all of these applications it is necessary to establish the normal levels and gender- and age-related changes of chemical elements in a large scale study of teeth.

Conflict of Interest

There is no any financial interest or any conflict of interest.

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