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Research article

Intracellular calcium excess as one of the main factors in the etiology of prostate cancer

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Abstract: Numerous studies show that prostate cancer (PCa) incidence drastically increases with age, these malignant tumours are mainly formed in the peripheral zone of the prostate gland, and a high intake of calcium rich dairy products is associated with an increased risk of PCa. The main objective of this study was to identify a potential common pathophysiological factor associated with the PCa features mentioned above. We performed measurements of the intracellular Ca concentrations in the peripheral zones of nonhyperplastic prostate glands of 99 males aged 0–87 years. To clarify the age-related changes in the intracellular Ca, a quantitative morphometric and two analytical methods of Ca determination were employed. We found, that in 18–45 years old males intracellular Ca was maintained at a relatively high concentration, which steadily increased with age. The intracellular Ca accumulation increased after the age of 45. We found, that by the age of 55, Ca level in the prostatic cells of the peripheral zone reached concentration, which is two-to-four-fold higher than in the 18 year olds. Age-dependent accumulation of Ca in the peripheral zone of human prostate gland has been previously unrecognized and could play an important role in the etiology of PCa.

Keywords: human prostate gland; peripheral zone; prostatic cells; prostatic fluid; calcium; aging; age-related changes in the human prostate; prostate cancer; dairy products

1. Introduction

Prostate cancer (PCa) is a global health problem that demands considerable attention as the disease is indolent, shows prolonged latency and PCa progression is associated with high morbidity and mortality [1,2]. In Europe and North America, PCa is the most common malignancy in men and is among the leading causes of death from cancer in males [3-5]. The American Cancer Society estimates that more than 2.9 million men in the United States live with prostate cancer, and 180,000 new cases are expected in 2016.

Due to the high incidence rates, carcinoma of the prostate is one of the most extensively studied malignancies. Despite this intensive international research PCa aetiology remains unclear. However its three hallmarks are well established: (i) the incidence rate of the PCa drastically increases with age, being three orders of magnitude higher for the age group 40–79 years than for those younger than 40 years [6,7]; (ii) about 80% of PCa tumours are formed in the peripheral zone of the prostate gland [8,9]; (iii) there is a positive association between the risk of PCa and consumption of dairy products [2,10-14].

The above well-established PCa features, when combined, allow us to hypothesize that there is an etiological factor(s) of malignant transformation, which may be located in, or selectively affects, the peripheral zone of the prostate gland. This effect increases with age and acts synergistically with a factor (or factors) linked to milk and dairy products consumption. Because milk and dairy products are the main source of Ca, Ca^{2+} intake for humans [11-15], we believe that dietary Ca could be such an etiological intra-prostatic factor.

Ca^{2+} is a key signaling molecule and is involved in a variety of fundamental biological processes from fertilization to programmed cell death. Almost every tissue and bodily liquid has specific functions that are controlled by Ca^{2+} and its spatio-temporal levels are tightly regulated by an extensive Ca^{2+}-signalling toolkit [16]. In particular, the prostatic gland differs markedly from other organs and soft tissues because of its relatively high Ca content [17,18]. For example, in the normal prostate Ca accumulates to up to seven-fold higher levels than in the liver and whole blood [19,20]. Further, prostatic Ca levels are age dependent, i.e. they increase from puberty to young adulthood [19-23] and then continue to increase until the age of 55–60 years [24-28].

Some literature reports Ca content in the prostate parenchyma. According to Deering et al. [29], prostatic parenchyma contains three main components: glandular epithelium (E), prostatic fluid contained in the glandular lumina (L), and fibromuscular tissue or stroma (S). In our previous study, we showed that Ca concentration in the prostatic fluid is almost two times higher than in the prostate parenchyma [23] and the prostatic fluid contained in the glandular lumina is the main pool of prostatic Ca in pediatric and nonhyperplastic young adult prostate glands [22,23]. Also, it was found that the relative volume of the prostatic fluid continues to increase for ages above 30, but the correlation between the Ca content in the prostatic parenchyma and the relative volume of the glandular lumina fades away [26-28,30,31]. These findings prompted us to investigate the age-related intracellular changes of Ca concentration in the prostate.

There are direct and indirect ways to investigate the prostatic intracellular Ca. The direct way includes measurement of Ca concentration in cells using imaging techniques. One of the oldest relevant imaging techniques is the X-ray microanalysis of freeze-dried cryosections [32]. Recently, the X-ray fluorescence (XRF) analysis of tissue Ca using a scanning beam of synchrotron radiation has been used for this purpose [33]. Although the synchrotron offers an extraordinary beam intensity and resolution, the sample preparation method, which can introduce uncertainties, remains a disadvantage

of the method. The technique requires cutting the tissue into thin sections and fixing the tissue samples using certain chemicals. During these procedures, some prostatic fluid is lost, and also Ca redistribution in prostate tissue takes place [34,35]. Thus, at best, the results of such studies can only give qualitative data and in the worst case give an erroneous Ca distribution.

In this study, an indirect but quantitative way of intracellular Ca estimation in the prostate gland was used. The primary purpose was to obtain data about the age-related changes in the morphometrics and Ca concentration in the peripheral zone of the non-hyperplastic prostate gland of healthy males. The second aim was to collect and assess published relevant reports on Ca concentration in the prostatic fluids, and the third aim was to evaluate the changes in the Ca concentration in the intracellular space of the prostate with age.

2. Materials and Methods

To clarify the age- dependent histological and Ca concentration changes in peripheral zones of nonhyperplastic prostate glands, a quantitative morphometric and two analytical methods of Ca determination were used. The prostates were obtained from autopsies of 99 subjects (European-Caucasian) aged 0–87 years who died mainly from sudden infant death syndrome, acute pulmonary etiologies (infants), pneumonia (children), and trauma (adults). All subjects were divided into the seven groups listed in Table 1, dependent on their age. The available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, neoplasm or other chronic disease that could affect the normal development of the prostate. None of the subjects was receiving medications known to affect prostate morphology, function or Ca content. All samples of the peripheral zone were divided by an anterior-posterior cross-section into two portions. One portion was reviewed by a histopathologist, while the other was used for the Ca concentration determination. Prostate glands containing any focus of benign prostatic hyperplasia, carcinoma, intraepithelial neoplasia, and slices with sample preparation artifacts were excluded from the study.

Morphometric evaluations were then performed quantitatively using a stereological method [36]. The mean volume fractions of the stroma (S), glandular epithelium (E), and glandular lumina (L) in prostate tissue were determined for each prostate specimen. Details of the stereological method and procedures used here were presented in our earlier publications concerning quantitative morphometric studies of the human prostate gland [22,23,27,28,30,31].

The samples intended for the Ca determination were weighed, freeze-dried and homogenized. Ca concentrations were estimated using instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and inductively coupled plasma atomic emission spectrometry (ICP-AES). There was a good agreement between the Ca levels determined by the two methods, and means of the two results from every sample were used in the study (Table 1). Details of the analytical methods and procedures used and appropriate details of relevant nuclear reactions, radionuclides, gamma-energies, wavelength, isotopes, spectrometers, spectrometer parameters and operating conditions have been presented in our earlier publications concerning the chemical elemental content of the human prostate gland [17,18]. For quality control of the results appropriate certified reference materials (CRM) including IAEA H-4 (animal muscle) were used [17,18,22,23,27,28,37].

Using the Microsoft Office Excel software, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for Ca concentrations in the peripheral zone of nonhyperplastic

prostate glands of males in the age range 0–87 years (seven age groups). The difference in the results between mean values of Ca concentration (mg/dm^3, wet mass basis) in prostate glands of the seven age groups was evaluated by Student's *t*-test. For the construction of the "Age dependence of intracellular Ca concentration in the peripheral zones of the prostate" diagram the Microsoft Office Excel software was also used.

Table 1. Mean (M ± SEM) values of Ca concentration (mg/dm^3, wet mass basis) in prostate glands of seven age groups obtained by two analytical methods.

Group No	Age (years)		*n*	NAA-SLR	ICPAES	Two methods combined
	Range	Mean				
Group 1	0–13	3.3	29	275 ± 34	234 ± 29	254 ± 31
Group 2	14–20	18.2	5	322 ± 53	284 ± 50	320 ± 75
Group 3	21–30	26.4	16	361 ± 45	401 ± 51	397 ± 52
Group 4	31–40	35.8	12	457 ± 48	457 ± 47	457 ± 48
Group 5	41–50	45.4	16	442 ± 47	433 ± 47	439 ± 47
Group 6	51–60	55.6	11	743 ± 188	763 ± 183	753 ± 185
Group 7	61–87	68.8	10	397 ± 34	416 ± 46	412 ± 46
All groups	0–87	31.0	99	405 ± 22	395 ± 32	404 ± 31

M: arithmetic mean, SEM: standard error of mean, NAA-SLR–instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides, ICPAES–inductively coupled plasma atomic emission spectrometry.

3. Results

Table 2 presents basic statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, and percentiles with 0.025 and 0.975 levels) of the Ca concentrations (mg/dm^3, wet mass basis) and the histologic components (fraction of dm^3, wet tissue) in the peripheral zone of nonhyperplastic prostate glands of males in the age range 0–13 years (group 1), 14–20 years (group 2), 21–30 years (group 3), and 31–40 years (group 4), 41–50 years (group 5), 51–60 years (group 6), and 61–87 years (group 7). In our previous publications, significant age-dependent changes of Ca content were found, and a good agreement was shown between our results and the medians of means for those age groups reported in the literature [22,23,27,28]. From results in the Tables 2 and 3 we can conclude that Ca concentration in the prostate parenchyma begins to increase after the age of puberty and by the age of 55 it is nearly 2.4 times higher than in the prostate glands of the 18-year-old males. Virtually the same trend can be seen for the relative volume of prostatic fluid, which equals to the relative volume of the glandular lumina.

There are several publications on the Ca concentration in prostatic fluid [38-43]. The median of the means of these concentrations agrees well with the finding by Kavanagh et al. [41]. The Ca concentration in prostatic fluid is strongly correlated to Zn [41] and because Zn concentration in the prostatic fluids is independent of age [44-47], age-independence of the Ca level might be implied. Thus, two relevant prostatic fluid characteristics have been taken from the literature and used in our subsequent calculations: (i) the mean Ca concentration in prostatic fluid equals 802 mg/dm^3 (802 mg/L) and (ii) Ca concentration does not vary with age. Using the Ca concentration in the prostatic

Table 2. Certain statistical characteristics of the Ca concentration (mg/dm^3, wet mass basis) and the histologic components (percent volumes) in the nonhyperplastic prostate gland of males aged 0–87 years.

Age group No. (Age range) Mean, years	Parameter	M	SD	SEM	Min	Max	Median	P0.025	P0.975
Group 1	Ca	254	152	31	73.7	671	205	78.9	598
(0–13 years)	Stroma	75.3	13.0	2.6	44.4	91.3	80.4	49.7	90.5
3.3	Epithelium	20.4	8.4	1.7	9.7	41.3	17.0	10.1	38.2
	Lumen	4.5	4.7	0.9	0.30	15.8	2.9	0.36	15.8
Group 2	Ca	320	130	75	183	441	336	191	436
(14–20 years)	Stroma	48.0	6.7	3.0	40.1	54.6	49.6	40.3	54.5
18.2	Epithelium	38.9	3.7	1.6	34.9	43.9	38.8	35.0	43.6
	Lumen	13.0	4.9	2.2	9.3	21.1	10.5	9.4	20.4
Group 3	Ca	397	186	52	214	764	331	219	763
(21–30 years)	Stroma	46.0	12.1	3.2	26.7	70.9	45.7	28.0	69.6
26.4	Epithelium	38.4	9.6	2.6	25.4	55.9	38.5	25.9	55.0
	Lumen	15.6	5.5	1.5	3.7	24.1	16.0	4.5	23.1
Group 4	Ca	457	151	48	239	692	470	251	673
(31–40 years)	Stroma	51.2	7.8	2.5	37.7	61.6	53.2	38.2	61.0
35.8	Epithelium	32.0	5.2	1.6	25.9	41.4	31.4	25.9	41.0
	Lumen	16.8	3.4	1.1	10.3	20.9	16.4	11.2	20.9
Group 5	Ca	439	155	47	245	725	373	248	690
(41–50 years)	Stroma	45.0	8.6	2.5	33.9	57.4	43.3	34.0	57.2
45.4	Epithelium	32.3	5.5	1.6	25.3	38.9	31.2	25.3	38.8
	Lumen	22.7	7.0	2.0	10.3	29.8	24.7	10.4	29.6
Group 6	Ca	753	556	185	291	2077	575	296	1859
(51–60 years)	Stroma	52.8	11.5	3.8	39.7	72.6	49.8	39.8	70.7
55.6	Epithelium	26.7	7.3	2.4	14.6	37.5	27.3	15.2	36.3
	Lumen	20.5	9.3	3.1	9.7	34.3	19.4	9.7	34.0
Group 7	Ca	412	130	46	261	656	420	265	627
(61–87 years)	Stroma	60.8	8.5	3.2	51.6	76.7	60.3	51.8	75.1
68.8	Epithelium	25.6	5.9	2.2	14.6	31.9	27.3	15.6	31.6
	Lumen	13.6	4.0	1.5	8.7	20.5	13.1	8.9	19.9
All groups	Ca	404	272	31	73.7	2077	346	93.7	960
(0–87 years)	Stroma	57.6	16.4	1.8	26.7	91.3	53.9	33.9	89.2
31.0	Epithelium	28.9	10.0	1.1	9.7	55.9	28.8	12.5	46.7
	Lumen	13.6	8.7	1.0	0.30	34.3	14.2	0.51	29.8

M: arithmetic mean, SD: standard deviation, SEM: standard error of mean, Min: minimum value, Max: maximum value, Med.: median, P0.025: percentile with 0.025 level, P0.975: percentile with 0.975 level.

fluid and values of relative prostatic fluid volume (L) (Table 2) we can calculate the Ca content of the prostatic fluid in 1 dm^3 of the wet prostatic tissue (Ca^L) for different age groups, as

$$Ca^{L}\ (mg) = 802\ (mg/dm^3)\ L\ (dm^3),$$

where L is the relative volume of the prostatic glandular lumina. If the prostate's peripheral zone is divided into two compartments comprising (a) fluid (L) and (b) solid tissue, or stromal (S) and epithelial (E) cells taken together, (S+E), then for these the relative volumes of (a) and (b) we can calculate Ca content of the prostatic cells in 1 dm^3 of wet peripheral zone prostatic tissue (Ca^{S+E}) for different age groups, as

$$Ca^{S+E}\ (mg) = Ca^{T}\ (mg) - Ca^{L}\ (mg),$$

where Ca^{T} is the Ca content of 1 dm^3 of wet prostatic parenchyma, because T the relative volume of the prostatic tissue equals 1 and T = S + E + L. Table 4 presents results of these calculations.

Before the next step of the calculation it is necessary to accept two alternative boundary conditions: Case 1 – Ca is uniformly distributed in all cells of the prostate (stromal and epithelial cells), or Case 2 – Ca is present only in the epithelial cells. Using the data in Table 4 for Case 1 we can calculate the intracellular Ca concentration for different age groups as

$$Ca^{\text{All cells}}\ (mg/dm^3) = Ca^{S+E}\ (mg)/[(S+E)\ (dm^3)],$$

and for Case 2 as

$$Ca^{\text{Epithelial cells}}\ mg/dm^3 = Ca^{S+E}\ (mg)/[E\ (dm^3)].$$

Figure 1 depicts the age-dependence of the intracellular Ca concentration for the Cases 1 and 2. In Case 1, the level of the intracellular Ca concentration increases very slowly in the age range from newborns to 45 years. In the age range from 18 to 45 years, it remains virtually constant and equals about 320 mg/dm^3. A marked increase in the intracellular Ca level begins after the age 45 years and reaches its maximum at about 55. This maximum equals 740 mg/dm^3 and is 2.3-fold higher than in the prostate glands of males aged 18 to 45 years.

In Case 2, the intracellular Ca level decreases in the age range from newborns to 18 years old and then slowly increases in males aged 18 to 45 years. Next, a marked increase of the intracellular Ca level begins after 45 years and reaches a maximum at about ten years later. Our data show that 55 years old males have intracellular Ca level in their epithelial cells of about 2200 mg/dm^3, which is

Table 3. Ratio of mean values (M) and the difference between mean values of Ca concentration in prostate glands of seven age groups (Student's *t*-test).

Ratio M_i/M_i (where I = 1–7)	M_1	M_2	M_3	M_4	M_5	M_6	M_7
M_1/M_i	1.00	0.79	0.64*	0.55**	0.58**	0.34*	0.61*
M_2/M_i	1.26	1.00	0.81	0.70	0.73	0.43	0.78
M_3/M_i	1.57*	1.24	1.00	0.87	0.90	0.53	0.96
M_4/M_i	1.81**	1.43	1.15	1.00	1.04	0.61	1.11
M_5/M_i	1.74**	1.37	1.11	0.96	1.00	0.58	1.07
M_6/M_i	2.98*	2.35	1.90	1.65	1.72	1.00	1.83
M_7/M_i	1.63*	1.29	1.04	0.90	0.94	0.55	1.00

M_i: arithmetic mean values of Ca mass fractions in the *i*-age group, *$p \leq 0.05$, **$p \leq 0.01$, statistically significant difference.

Table 4. Mean values of Ca contents in all prostatic cells of peripheral zones, contained in 1 dm^3 of wet prostatic tissue for the different age groups.

Group No	Mean age years	Ca^T mg	L dm^3	E dm^3	S+E dm^3	Ca^L mg	Ca^{S+E} mg
Group 1	3.3	254	0.045	0.203	0.955	36.1	217,9
Group 2	18.2	320	0.131	0.389	0.869	105	214,9
Group 3	26.4	397	0.156	0.384	0.844	125	271,9
Group 4	35.8	457	0.168	0.320	0.832	135	322,3
Group 5	45.4	439	0.227	0.323	0.773	182	256,9
Group 6	55.6	753	0.205	0.267	0.795	164	588,6
Group 7	68.8	412	0.136	0.256	0.864	109	302,9

Ca^T: Ca content in 1 dm^3 of wet prostatic tissue,

L: volume fraction of lumen (prostatic fluid) in 1 dm^3 of wet prostatic tissue,

S: volume fraction of stroma (stromal cells) in 1 dm^3 of wet prostatic tissue,

E: volume fraction of epithelium (epithelium cells) in 1 dm^3 of wet prostatic tissue,

(S+E): volume fraction both of stromal and epithelium cells taken together in 1 dm^3 of wet prostatic tissue,

Ca^L: Ca of prostatic fluid in 1 dm^3 of wet prostatic tissue,

Ca^{S+E}: Ca of all prostatic cells in 1 dm^3 of wet prostatic tissue

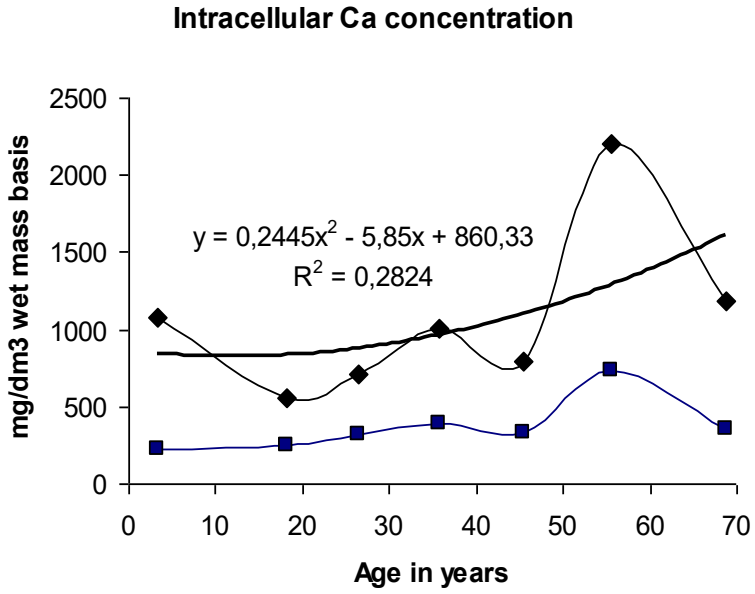

Figure 1. Age dependence of intracellular Ca concentration (mg/dm^3, wet mass basis) in the peripheral zones of the prostate: Case 1. (■) age dependence of intracellular Ca concentration in the prostate, presuming there is uniform distribution of Ca in both stromal and epithelial cells, Case 2. (♦) age dependence of intracellular Ca concentration in epithelial cells, presuming there is an accumulation of Ca only in the epithelial cells, and the trend curve.

four times higher than in the prostate glands of 18-year-old men. Because both the prostate epithelial cells and cells of the stromal component need Ca for their normal function the real changes in the prostatic intracellular Ca concentrations at different ages lie somewhere between Case 1 and Case 2. Either way, an excessive accumulation of Ca in prostatic cells begins after 45 culminating in the two-to-four fold increase in the intracellular Ca levels by the age of 55 years.

4. Discussion

As was shown by us [17,19,20] the use of IAEA H-4 (animal muscle) as a certified reference material for the analysis of samples of prostate tissue can be seen as acceptable. Good agreement of the Ca content analyzed by INAA-SLR (238 ± 59 mg/kg, Mean ± SD) with the certified data of IAEA H-4 (163–213 mg/kg, 95% confidence interval) indicated an acceptable accuracy of the results obtained in the study of Ca content in the prostate presented in Tables 1–4.

This study augments our current understanding of prostate gland physiology. Firstly, this study shows that the intracellular Ca concentration in the 18 to 45 year olds remains almost constant at a relatively high level. In these men, the average intracellular Ca in the prostate glands does not increase beyond 760 mg/dm^3. Such a high level of Ca in the prostate cells is four-to-five times higher than mean values for Ca in other organs of the human body including skeletal muscle (70–150 mg/dm^3), liver (50–130 mg/dm^3), lung (80–200 mg/dm^3), and kidney (100–190 mg/dm^3) [48]. Since the function of the prostate gland in the age range between 18 to 45 years is presumably normal, we must conclude that a specific accumulation of Ca by the epithelial cells of the prostate gland is a normal physiological event.

Secondly, our work demonstrates that after the age of 45 the intracellular Ca level in the prostate glands keeps increasing and reaches 740–2200 mg/dm^3, which is at least an order of magnitude higher than Ca concentrations in most other tissues. Our results are in line with the results of by Tvedt et al. [32], who demonstrated high cytosolic Ca concentrations in the human prostatic cells (700 mmol/kg dry mass basis or approximately 5600 mg/dm^3 wet mass basis) and an age-related increase of intranuclear Ca.

According to the well-established hypothesis of Costello and Franklin, the main functions of the prostate secretory epithelial cells are production, accumulation, and secretion of a large amount of citrate [49]. In the prostate, intracellular citrate level could reach 1800 mmol/kg [50] and high intracellular Ca level along with other major electrolytes and Zn may be needed for the ionic equilibrium in the cytosol and extracellular prostatic fluid. On the other hand, Ca^{2+} is one of the most ubiquitous and critical secondary messengers and is involved in a plethora of cellular processes [51,52]. The role of Ca^{2+} is well established in the majority of cell signaling pathways involved in carcinogenesis such as cellular motility, proliferation, and apoptosis [52-56].

In particular, intracellular transmission of the Ca^{2+} signal occurs via Ca^{2+} sensor proteins such as regucalcin (RGN), calmodulin (CaM) and related members of the Ca^{2+} binding protein family. The $Ca^{2+/}$CaM complex regulates over 120 proteins, including transcription factors, channels, pumps, kinases and phosphatases [57]. A rise in intracellular calcium promotes the $Ca^{2+/}$CaM complex, which targets the CaM dependent kinase that mediate nutrient responses and regulates cell growth and energy balance. Recent work of Dubois et al. [58] demonstrated that through the differential expression of specific ORAI Ca^{2+} channels prostate cancer cells promote the store-independent Ca^{2+} influx across the plasma membrane, which results in enhanced proliferation and apoptotic resistance.

In various types of tissues including breast and prostate, Ca^{2+} homeostasis is maintained by the Ca-binding protein regucalcin (RGN). RGN is conserved in vertebrates and regulates intracellular Ca^{2+} homeostasis through the modulation of the activity of Ca^{2+} channels, Ca^{2+}-ATPase in the membrane of the mitochondria and endoplasmic reticulum [59] and (Ca^{2+}-Mg^{2+})-ATPase in the plasma membrane [60]. The expression of RGN is controlled by intracellular Ca^{2+} and regulatory transcription factors, and was identified as a target gene for sex steroid hormones in the prostate glands [61]. RGN is down-regulated in prostate cancer tissues and Maia et al. have demonstrated, that RGN immunoreactivity is correlated with the grade of adenocarcinoma cellular differentiation [62]. Notably, in rat brain neurons, the RGN inhibitory effect on Ca^{2+}-ATPase is weakened with aging. Therefore by analogy to the brain tissue age-related or malignant transformation related down-regulation of RGN in the background of increasing intracellular and extracellular Ca concentrations may play a relevant role in cancer initiation and progression in the prostate [63].

Further, Ca^{2+} channels in the plasma membrane of neurons are critical for the basal activity of the ubiquitous transcription factor that controls cell proliferation and survival NF-κB [56]. Galheigo et al. have recently shown that in chemically induced mice NF-κB stimulated prostate carcinogenesis, which provides another link between proliferation and Ca influx [64]. In addition, Ca^{2+} signalling is critical for regulating cell migration and invasion and a number of Ca permeable channels have been implicated in the enhanced migration of cancer cells [65].

Another aspect of the prostate cancer biology where intracellular Ca overload is may be involved, but has not yet been fully explored, is the cellular energy metabolism. For example, Panov and Orynbaeva have suggested that due to the 20–30 mV higher electrical membrane potential in prostate cancer cell lines, mitochondrial metabolism of the metastatic prostate cancer cells is predominantly based on utilization of glutamate and glutamine, potentially resulting in cachexia [66].

Overall, excessive intracellular, intranuclear, and extracellular Ca concentrations make the peripheral zone of the prostate prone to tumorigenesis. A continuously increasing intracellular Ca level could disturb the fine-tuned Ca^{2+} homeostasis and together with the aging effects could trigger a cascade of events resulting in tumor initiation and progression. Almost certainly, a Ca rich diet would contribute to Ca accumulation in the prostate and may support abnormal tissue growth. But the molecular mechanisms of the accumulation are yet to be discovered.

This study has several limitations. Firstly, analytical techniques employed in this study measure bulk Ca and do not differentiate between Ca ions and elemental Ca. Secondly, the methods we used do not provide information on the spatial distribution of Ca in the cell. Nonetheless, our data are crucial for the understanding of Ca homeostasis in the prostate gland and highlight the need for more studies addressing the unique Ca rich intracellular and extracellular environment in the prostatic gland.

5. Conclusion

At rest, the cytosolic concentration of Ca2+ in eukaryotic cells is maintained in nanomolar ranges. In contrast, the prostate gland accumulates and secretes large amounts of Ca and must have evolved mechanisms to control this ubiquitous messenger. Age-dependent increase in the Ca level in the peripheral zone of the prostate and particularly the accumulation of this element in the prostatic intracellular space at age over 45 years were found in this study. This increase may override these protection mechanisms and promote malignancy in this part of the prostate, which is common in aging men and was shown to have a positive association with a Ca rich diet.

Conflict of interest

The authors declare no conflict of interest.

References

1. Forootan SS, Hussain S, Aachi V, et al. (2014) Molecular mechanisms involved in the transition of prostate cancer cells from androgen dependent to castration resistant state. *J Androl Gynaecol* 2: 9.
2. Tao ZQ, Shi AM, Wang KX, et al. **(**2015) Epidemiology of prostate cancer: current status. *Eur Rev Med Pharmacol Sci* 19: 805-812.
3. Center AA, Jemal A, Lortet-Tieulent J, et al. (2012) International variation in prostate cancer incidence and mortality rates. *Eur Urol* 61: 1079-1092.
4. Cohen LA (2002) Nutrition and prostate cancer: a review. *Ann NY Acad Sci* 963: 148-155.
5. Jones BA, Liu W-L, Araujo AB, et al. (2008) Explaining the race difference in prostate cancer stage at diagnosis. *Cancer Epidemiol Biomarkers Prev* 17: 2825-2834.
6. Jemal A, Murray T, Samuels A, et al. (2003) Cancer statistics, 2003. *CA Cancer J Clin* 53: 5-26.
7. Rebbeck TR (2006) Conquering cancer disparities: new opportunities for cancer epidemiology, biomarker, and prevention research. *Cancer Epidemiol Biomarkers Prev* 15: 1569-1571.
8. Strahan RW (1963) Carcinoma of the prostate: Incidence, origin, pathology. *J Urol (Baltimore)* 89(6): 875-880.
9. McNeal JE, Redwine EA, Freiha FS, et al. (1988) Zonal distribution of prostatic adenocarcinoma. *Am J Surg Pathol* 12: 897-906.
10. Giovanucci E, Ascherio A, Rimm E, et al. (1995) Intake of carotenoids and retinol in relation to risk of prostate cancer. *J Natl Cancer Inst* 87: 1767-1776.
11. Song Y, Chavarro JE, Cao Y, et al. **(**2013) Whole milk intake is associated with prostate cancer-specific mortality among U.S. male physicians. *J Nutr* 143: 189-196.
12. Aune D, Rosenblatt DAN, Chan DS, et al. (2015) Dairy products, calcium, and prostate cancer risk: a systematic review and meta-analysis of cohort studies. *Am J Clin Nutr* 101: 87-117.
13. Lin PH, Aronson W, Freedland SJ (2015) Nutrition, dietary interventions and prostate cancer: the latest evidence. *MC Med* 13: 3.
14. Wilson KM, Shui IM, Mucci LA, et al. **(**2015) Calcium and phosphorus intake and prostate cancer risk: a 24-y follow-up study. *Am J Clin Nutr* 101: 173-183.
15. Zaichick V, Tsyb A, Matveenko E, et al. (1996) Instrumental neutron activation analysis of essential and toxic elements in the child and adolescent diets in the Chernobyl disaster territories of the Kaluga Region. *Sci Total Environ* 192: 269-274.
16. Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 4: 517-529.
17. Zaichick S, Zaichick V (2011) INAA application in the age dynamics assessment of Br, Ca, Cl, K, Mg, Mn, and Na content in the normal human prostate. *J Radioanal Nucl Chem* 288: 197-202.
18. Zaichick V, Nosenko S, Moskvina I (2012) The effect of age on 12 chemical element contents in intact prostate of adult men investigated by inductively coupled plasma atomic emission spectrometry. *Biol Trace Elem Res* 147: 49-58.

19. Zaichick V, Zaichick S (2013) The effect of age on Br, Ca, Cl, K, Mg, Mn, and Na mass fraction in pediatric and young adult prostate glands investigated by neutron activation analysis. *Appl Radiat Isot* 82: 145-151.
20. Zaichick V, Zaichick S (2013) NAA-SLR and ICP-AES Application in the assessment of mass fraction of 19 chemical elements in pediatric and young adult prostate glands. *Biol Trace Elem Res* 156: 357-366.
21. Zaichick V, Zaichick S (2014) Androgen-dependent chemical elements of prostate gland. *Androl Gynecol Curr Res* 2: 2.
22. Zaichick V, Zaichick S (2014) Relations of the neutron activation analysis data to morphometric parameters in pediatric and nonhyperplastic young adult prostate glands. *Adv Biomed Sci Eng* 1: 26-42.
23. Zaichick V, Zaichick S (2014) Relations of the Al, B, Ba, Br, Ca, Cl, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, and Zn mass fractions to morphometric parameters in pediatric and nonhyperplastic young adult prostate glands. *BioMetals* 27: 333-348.
24. Zaichick V, Zaichick S (2014) INAA application in the assessment of chemical element mass fractions in adult and geriatric prostate glands. *Appl Radiat Isot* 90: 62-73.
25. Zaichick V, Zaichick S (2014) Determination of trace elements in adults and geriatric prostate combining neutron activation with inductively coupled plasma atomic emission spectrometry. *Open J Biochem* 1: 16-33.
26. Zaichick V (2015) The variation with age of 67 macro- and microelement contents in nonhyperplastic prostate glands of adult and elderly males investigated by nuclear analytical and related methods. *Biol Trace Elem Res* 168: 44-60.
27. Zaichick V, Zaichick S (2016) Age-related changes in concentration and histological distribution of Br, Ca, Cl, K, Mg, Mn, and Na in nonhyperplastic prostate of adults. *Eur J Biol Med Sci Res* 4: 31-48.
28. Zaichick V, Zaichick S (2016) Age-related changes in concentration and histological distribution of 18 chemical elements in nonhyperplastic prostate of adults. *World J Pharm Med Res* 2: 5-18.
29. Deering RE, Choongkittaworn M, Bigler SA, et al. (1994) Morphometric quantitation of stroma in human benign prostatic hyperplasia. *Urology* 44: 64-67.
30. Zaichick S, Zaichick V (2013) Relations of morphometric parameters to zinc content in paediatric and nonhyperplastic young adult prostate glands. *Andrology* 1: 139-146.
31. Zaichick V, Zaichick S (2014) Age-related histological and zinc content changes in adult nonhyperplastic prostate glands. *Age* 36: 167-181.
32. Tvedt KE, Kopstad G, Haugen OA, et al. (1987) Subcellular concentrations of calcium, zinc, and magnesium in benign nodular hyperplasia of the human prostate: X-ray microanalysis of freeze-dried cryosections. *Cancer Res* 47: 323-328
33. Banas A, Banas K, Kwiatek WM, et al. (2011) Neoplastic disorders of prostate glands in the light of synchrotron radiation and multivariate statistical analysis. *J Biol Inorg Chem* 16: 1187-1196.
34. Zaichick V, Zaichick S (1996) Instrumental effect on the contamination of biomedical samples in the course of sampling. *J Anal Chem* 51: 1200-1205.
35. Zaichick V (1997) Sampling, sample storage and preparation of biomaterials for INAA in clinical medicine, occupational and environmental health. In: *Harmonization of Health-Related Environmental Measurements Using Nuclear and Isotopic Techniques*, Vienna: IAEA, 123-133.
36. Avtandilov GG (1973) *Morphometry in pathology*, Moscow: Medicina.

37. Zaichick V (1995) Applications of synthetic reference materials in the medical Radiological Research Centre. *Fresenius J Anal Chem* 352: 219-223.
38. Burgos MH (1974) Biochemical and functional properties related to sperm metabolism and fertility. In: Brandes D., Ed, *Male accessory sex organs*. New York: Academic press, 151-160.
39. Homonnai ZT, Matzkin H, Fainman N, et al. (1978) The cation composition of the seminal plasma and prostatic fluid and its correlation to semen quality. *Fertil Steril* 29: 539-542.
40. Zaneveld LJD, Tauber PF (1981) Contribution of prostatic fluid components to the ejaculate. In: Murphy G.P., Sandberg A.A., Karr J.P., Eds. *Prostatic Cell: Structure and Function*. New York: Alan R. Liss, Part A, 265-277.
41. Kavanagh JP, Darby C, Costello CB, et al. (1983) Zinc in post prostatic massage (VB_3) urine samples: a marker of prostatic secretory function and indicator of bacterial infection. *Urol Res* 11: 167-170.
42. Daniels GF, Grayhack JT (1990) Physiology of prostatic secretion. In: Chisholm G.D., Fair W.R., Eds. *Scientific Foundation in Urology*. Chicago: Heinemann Medical Books, 351-358.
43. Romics I, Bach D (1991) Zn, Ca and Na levels in the prostatic secretion of patients with prostatic adenoma. *Int Urol Nephrol* 23: 45-49.
44. Mackenzie AR, Hall T, Whitmore WFJr (1962) Zinc content of expressed human prostate fluid. *Nature* 193: 72-73.
45. Fair WR, Cordonnier JJ (1978) The pH of prostatic fluid: A reappraisal and therapeutic implications. *J Urol* 120: 695-698.
46. Kavanagh JP (1983) Zinc binding properties of human prostatic tissue, prostatic secretion and seminal fluid. *J Reprod Fert* 68: 359-363.
47. Zaichick V, Sviridova T, Zaichick S (1996) Zinc concentration in human prostatic fluid: normal, chronic prostatitis, adenoma and cancer. *Int Urol Nephrol* 28: 687-694.
48. Iyengar GV, Kollmer WE, Bowen HGM (1978) *The Elemental Composition of Human Tissues and Body Fluids. A Compilation of Values for Adults*. Weinheim: Verlag Chemie.
49. Costello LC, Franklin RB (2009) Prostatic fluid electrolyte composition for the screening of prostate cancer: a potential solution to a major problem. *Prostate Cancer Prostate Dis* 12: 17-24.
50. Costello LC, Lao L, Franklin R (1993) Citrate modulation of high-affinity aspartate transport in prostate epithelial cells. *Cell Mol Biol* 39: 515-524.
51. Roderick HL, Cook SJ (2008) Ca^{2+} signalling checkpoints in cancer: remodelling Ca^{2+} for cancer cell proliferation and survival. *Nat Rev Cancer* 8: 361-375.
52. Flourakis M, Prevarskaya N (2009) Insights into Ca^{2+} homeostasis of advanced prostate cancer cells. *Biochim Biophys Acta* 1793: 1105-1109.
53. Feng M, Grice DM, Faddy HM, et al. (2010) Store-independent activation of orai1 by SPCA2 in mammary tumors. *Cell* 143: 84-98.
54. Yang H, Zhang Q, He J, et al. (2010) Regulation of calcium signaling in lung cancer. *J Thorac Dis* 2: 52-56.
55. Berridge MJ, Lipp P, Bootman MD (2000) The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* 1: 11-21.
56. Monteith GR, Davis FM, Roberts-Thomson SJ (2012) Calcium channels and pumps in cancer: changes and consequences. *J Biol Chem* 287: 31666-31673.
57. Marcelo KL, Means AR, York B (2016) The Ca^{2+}/Calmodulin/CaMKK2 Axis: Nature's Metabolic CaMshaft. *Trends Endocrinol Metab* 27: 706-718.

58. Dubois C, Vanden Abeele F, Lehen'kyi V, et al. (2014) Remodeling of channel-forming ORAI proteins determines an oncogenic switch in prostate cancer. *Cancer Cell* 26: 19-32.
59. Yamaguchi M, Mori S (1989) Activation of hepatic microsomal Ca2+-adenosine triphosphatase by calcium-binding protein regucalcin. *Chem Pharm Bull* 37: 1031-1034.
60. Takahashi H, Yamaguchi M (1993) Regulatory effect of regucalcin on (Ca(2+)-Mg2+)-ATPase in rat liver plasma membranes: comparison with the activation by Mn2+ and Co2+. *Mol Cell Biochem* 124: 169-174.
61. Laurentino SS, Correia S, Cavaco JE, Oliveira PF, Rato L, et al. (2011) Regucalcin is broadly expressed in male reproductive tissues and is a new androgen-target gene in mammalian testis. *Reproduction* 142: 447-456.
62. Maia C, Santos C, Schmitt F, Socorro S (2009) Regucalcin is under-expressed in human breast and prostate cancers: effect of sex steroid hormones. *J Cell Biochem* 107: 667-676.
63. Yamaguchi M (2012) Role of regucalcin in brain calcium signaling: involvement in aging. *Integr Biol (Camb)* 4: 825-837.
64. Galheigo MR, Cruz AR, Cabral ÁS, et al. (2016) Role of the TNF-α receptor type 1 on prostate carcinogenesis in knockout mice. *Prostate* 76: 917-926.
65. Prevarskaya N, Ouadid-Ahidouch H, Skryma R, et al. (2014) Remodelling of Ca^{2+} transport in cancer: how it contributes to cancer hallmarks? *Philos Trans R Soc Lond B Biol Sci* 369: 20130097.
66. Panov A, Orynbayeva Z (2013) Bioenergetic and antiapoptotic properties of mitochondria from cultured human prostate cancer cell lines PC-3, DU145 and LNCaP. *PLoS One* 8: e72078.

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