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14. ABSTRACT The purpose of this study is to examine associations between zinc (Zn), selenium (Se) and cadmium (Cd), on development of prostate cancer. The objectives of this project are: 1) to establish the reliability of using formalin-fixed paraffin-embedded (FFPE) prostate tissue by comparing levels of Zn, Se and Cd between FFPE and fresh-frozen unfixed prostate tissues; 2) to enhance our knowledge of prostate cancer environmental etiology by examining the association of Cd, Zn, Se with the development and progression of cancer using FFPE tissues; 3) to enhance the use of the AFIP National Tissue Repository of archival tissues. First objective has been successfully accomplished based on the development of a method which uses high-resolution inductively-coupled mass-spectrometry (ICP-MS) for the analysis of Zn, Cd, Se including arsenic (As) and iron (Fe) in FFPE prostate tissue. Our results have demonstrated that in comparison with fresh tissue, Fe, Se and As do not show much difference in the FFPE tissue. On the other hand, Zn and Cd resulted in ~40% and ~60% loss, respectively, requiring using the correction factors to assess their content in the original tissue. The technique can be useful in various studies for analysis of archival FFPE tissue, and provides a basis for fulfilling the next aim of the project, the analysis of the above elements in FFPE prostate tissue of patients with different relation to cancerogenesis.						
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Project Progress Report

Introduction.

Prostate cancer is most frequently diagnosed form of cancer and the second leading cause of cancer death in USA men. A better understanding prostate cancer etiology, with focus on protective and aggravating factors may facilitate preventative or prospective measures to be taken to reduce morbidity and mortality from this disease. Three environmental trace elements, zinc (Zn), selenium (Se) and cadmium (Cd), have been highlighted in the literature in relation to the development of prostate disease (1, 2). Zinc (Zn) is second in abundance metal in human body that serves as cofactor of more than 300 enzymes participating in various biochemical reactions. In prostate, Zn is accumulated up to 10-fold higher levels (~150 µg/g) than in any other organ, and it is require for a healthy state of the gland (3), whereas several-fold decrease have been observed in the malignant tissue (4-6). Cadmium (Cd) possesses carcinogenic effect that is hormonally mediated (7, 8), and is recognized as a risk factor in development of prostate cancer (2, 9-11). In prostate, cadmium has demonstrated a distinct antagonist effect with Zn, presumably due to competing for binding sites with the same ligands in cell (12, 13). Selenium (Se) , a component of several amino acid (selenocysteine) and proteins (selenoproteins) (14), serves as antioxidant protectors (15) and is involved in DNA reparation (16). Studies have demonstrated an inverse correlation between environmental Se levels and prostate cancer mortality (17, 18) and established beneficial role of Se in reducing risk of cancer, where Se can interfere with carcinogenic activity of Cd (4, 19-20). The general purpose of the current project is to examine association between Zn, Se and Cd on the development of prostate cancer that remains unclear or under-researched.

The current investigation is an extension of our preliminary study (21). Based on the success of this preliminary work, the current project is aimed at the development of methodology for the analysis of levels of Zn, Se and Cd in formalin-fixed paraffin-embedded (FFPE) prostate tissue (objective 1), followed by the analysis of clinically and histologically well-characterized archival materials for possible correlations of these metals' levels with the presence of cancer and its aggressiveness (objective 2).

Description of project accomplishment.

In recent investigations, we demonstrated existence of links and ethnic differences between elevated Prostate-Specific Antigen (PSA) and Cd, Zn and Se exposure and their blood levels (Gray et al, (21)). It was found a positive association between blood Cd and total PSA ($p < 0.001$, $r = 0.226$), which remained significant within multivariate model. The mean PSA level in low blood Cd level category was 0.85 ng/ml (95% CI 0.12-1.33 ng/ml), in the medium category it was 0.90 ng/ml (95% CI 0.09-1.23 ng/ml) and in the high category 1.64 ng/ml (95% CI 0.40-2.52 ng/ml). There was a significant difference in PSA levels between the low and the high blood Cd levels ($p < 0.001$), but no association was found between Se and Zn levels and elevated PSA (21).

In current investigation, initial training was performed to master the technique of high resolution inductively coupled plasma mass-spectrometry analysis (ICP-MS) of trace metals in materials of different nature including biological tissues. During the course of training, the samples were prepared for analysis employing a digestion technique based on the use of microwave-accelerated digestion system (MARS-X and MARS-5 microwave ovens (CEM Corp., Matthews, NC) using nitric acid and hydroxide peroxide. The training was performed on ICP-MS machine Thermo Finnigan Element 2 (Thermo Electron Corp., Bremen, Germany).

Our next step was to explore possibility of using FFPE tissue to assess the levels of Cd, Zn and Se in the original fresh tissue. In this panel, we also included additional elements, Fe and As, known to participate in prostate cancerogenesis. One of the most important questions to be answered was if procedure of embedding tissue in paraffin leads to loss of the metals; therefore, we performed comparison of the metals' levels between FFPE tissue and the matching fresh tissue. At first step, we established optimal experimental conditions for ICP-MS analyses using a number of standard reference materials representing biological tissues of different origin, NIST Liver-1577b, DOLT-3, DORM-2 and TORT-3 (purchased from NIST (Gaithersburg, MD) and National Research Council of Canada (Ottawa, Ontario, Canada)), respectively, with the certified levels of elements of our interest. The reference materials were analyzed using ~150 mg of the tissue that is close to the amounts of corresponding tissue specified by the manufacturers for certification of the metals' levels. The samples were digested with nitric acid in standard Teflon vials (MARSXpress™ D, CEM Corp.) in microwave-accelerated reaction at 180 °C for 30 min and analyzed by a sector-field high resolution

inductively coupled plasma with mass spectrometry (ICP-MS). In these experiments, we used yttrium (Y) and ⁷⁴Se as internal standards and corresponding standard solutions of Fe, Se, As, Zn and Cd for quantification. In Table 1 are summarized conditions used for high resolution ICP-MS analysis of Fe, Se, As, Zn and Cd in the samples.

Table 1. *Operating conditions for ICP-MS analysis of Fe, Zn, As, Se and Cd.*

ICP operating parameters setting										
Plasma power	1550 W		Lenses	High resolution lenses						
Auxiliary gas flow	0.8 L min ⁻¹		Extraction (V)	-2000		Rotation quad 1 (V)		-1.03		
Sample gas flow	1.05 L min ⁻¹		Focus (V)	-800		Focus quad 1 (V)		-2.31		
Additional gas 1 flow	0.14 L min ⁻¹		X-deflection (V)	-9.5		Rotation quad 2 (V)		-2.8		
Sampler/skimmers cones	Nickel		Y-deflection (V)	-6.51						
Take-up time	1.5 min		Shape (V)	115						
Mass-spectrometer acquisition parameters setting										
Isotope	Mass	Mass range	Magnet Mass	Settling time	Sample Time	Segment	Detect mode	IS index	IS name	Internal stand
⁵⁷ Fe	56.9349	56.932 - 56.938	56.935	0.3	0.01	0.3	Both	7	Y-89	
⁶⁶ Zn	65.9255	65.922 - 65.929	65.925	0.034	0.01	0.3	Both	7	Y-89	
⁷⁴ Se	73.9219	73.918 - 73.926	65.925	0.001	0.01	0.3	Both			yes
⁷⁵ As	74.9211	74.917 - 74.925	65.925	0.001	0.03	0.9	Counting	3	Se-74	
⁷⁶ Se	75.9187	75.915 - 75.922	75.919	0.034	0.03	0.9	Counting	3	Se-74	
⁷⁷ Se	76.9194	76.916 - 76.923	75.919	0.001	0.03	0.9	Counting	3	Se-74	
⁸⁹ Y	88.9053	88.901 - 88.910	88.905	0.036	0.01	0.3	Counting			yes
¹¹¹ Cd	110.9036	110.898 - 110.909	110.904	0.041	0.01	0.3	Counting	7	Y-89	
Mass window	100									
Samples per peak	30									
Search window	60									
Integration window	20									
Scan type	Escan									
Integration type	Average									
Regression type	Linear									

Generally, the detection limits (defined as triplicated standard deviation values for each element) for analysis of blank solutions (containing the same solvent composition including internal standards) for the above metals were in the range of 0.01-1 µg/L that is far below the range of physiological levels of these metals in tissues. The detected levels of the metals in the standard materials were found to be within 10-15% range of the corresponding certified values and the precision (relative standard deviation of the measurements) were 0.5-13% that confirmed reliability of the used technique. During these experiments, we also tested the effect of introducing acetic acid to the solutions analyzed by ICP-MS. We found that the addition of acetic acid up to 3% is resulted on an increase of As and Se signals by up to 2.5-fold as reflected by a ~2-fold improvement on detection limits and higher precision. Thus, in further experiments we used acetic acid added up to 3% to the digested tissue samples. These results are demonstrated in Table 2.

Table 2. Detection limits ($\mu\text{g/L}$) and precision (RSD, %) for Fe, Zn, As, Se and Cd determination in standard materials using ICP-MS. The dried tissue samples (~ 150 mg), taken in triplicates, were digested with 70% nitric acid at 180°C and analyzed by high-resolution ICP-MS as described within text. In brackets shown values obtained when 3% of acetic acid was added to each sample.

	Fe	Zn	As	Se	Cd
Detect. Limit ($\mu\text{g/L}$)	1.61 (1.25)	0.64 (0.80)	0.04 (0.02)	0.08 (0.04)	0.01 (0.01)
Nist Liver (RSD, %)	0.3 (2.6)	1.1 (1.5)	13 (11.5)	4.0 (2.3)	1.7 (3.4)
DOLT-3 (RSD, %)	1.1 (0.9)	1.0 (2.1)	0.9 (1.5)	0.5 (1.4)	0.8 (1.8)
DORM-2 (RSD, %)	2.8 (3.1)	4.9 (2.7)	1.0 (0.8)	1.7 (0.9)	2.7 (11.8)
TORT-2 (RSD, %)	3.6 (3.5)	1.9 (2.5)	1.4 (1.2)	1.4 (0.9)	1.0 (1.5)

Since the analysis of tissue recovered from paraffin blocks requires using significantly smaller amounts of the tissue (5-15 mg), we next tested if we were able to obtain comparable results analyzing the standard materials at such small-scale amounts. Approximately ~ 15 mg of the certified materials were digested in Teflon 3-mL Micro Vessels D (CEM Corp.) under 110°C , and analyzed by ICP-MS. Under these conditions, the obtained values of precision and concentrations of the elements of interest were similar to the corresponding values obtained in former experiments (Table 2), validating the use of micro-scale technique. The typical result of analysis of one of standard materials, NIST liver, is shown in Fig. 1A.

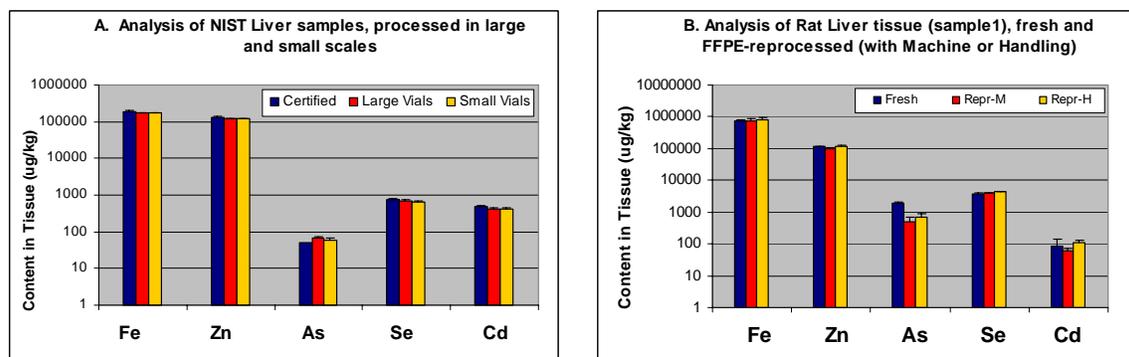


Fig. 1. Analyses of selected samples of certified reference material NIST Liver and rat liver for levels of Fe, Zn, Se, As and Cd. The dried tissue samples (~ 150 mg) were digested with 70% nitric acid in microwave-accelerated reaction, and analyzed by high-resolution ICP-MS as described within text. Experiments were performed in triplicates, and the errors are shown in vertical bars. In panel A, (NIST Liver) in blue are shown the values of metal's concentrations and corresponding standard deviations (shown in vertical bars) provided by the manufacturer. In red and yellow are presented the results for samples digested in the large and small sample size scales, respectively. In panel B (rat liver), the dried fresh tissue sample is shown in blue, the sample reprocessed (deparaffinized) using machine-based procedure is marked as Repr-M (shown in red), and the sample reprocessed with handling is marked as Repr-H (yellow).

During this study, we also optimized technique for paraffin extraction, assuming that this process having the same chemistry as embedding step does not affect markedly the tissue quality. As a model of human tissue, for initial experiments we used rat liver, based on preliminary data with FFPE mice liver and kidney that indicated good recovery of elements of the interest (unpublished data). The rat liver tissue samples obtained from the same and different animals were divided in three sections each, which were analyzed by ICP-MS, directly (on dry tissue basis) or after standard automated paraffin-embedding (with tissue processor) and its removal using either an automated (using xylene) or handling-based (using hexane) extraction. We found that in comparison with fresh tissue, the recovery of Fe, Zn, Se and Cd from FFPE rat liver occurred at the same high levels (90-100%), whereas the recovery of As was about 20%. Besides possible leakage of the metals, the difference in their levels between fresh and FFPE tissue may be explained by uneven distribution of the elements within the same tissue (as observed for all the metals in samples taken from the same animal), and also by a change in dry tissue weight due to extraction of some components (i.e. lipids) during the embedding process. High recovery of Fe is in good agreement with previous studies of other authors demonstrating about 100% recovery of Fe from FFPE human liver tissue (22). We also found that both techniques of paraffin removal produce comparable values of the metals' concentrations, thus favoring the automated process as a faster and less laborious procedure. The typical results of analysis of a selected sample are illustrated in Fig. 1B.

Finally, we studied recovery of the metals in FFPE prostate tissue in comparison with that from matching un-fixed fresh tissue. The tissue specimen was obtained from a single case of radical prostatectomy performed for prostate cancer, taken distantly from a localized cancer focus. These specimens were a courtesy of our group's collaborator Dr. Andre Kajdacsy-Balla (University of Illinois), who performed the clinical and morphological evaluation of the tissues. Tissue specimens were received in frozen un-fixed state, de-identified from the NIH-sponsored Prostate Cancer Tissue Resource (<http://cpctr.cancer.gov>) and kept under -80°C until ready to be analyzed. Seven portions of the tissue were taken from the area not having an evidence of hypertrophy and analyzed for the metals' of interest, directly and after paraffin-embedding and its removal with automated or handling-based process as discussed above. Both techniques of the paraffin extraction resulted in detection of similar levels of the metals in the tissue, validating the data and supporting the use of automated technique as most practical. In

comparison with the fresh tissue, the recovery of metals was found to have some differences. Generally, Fe, Se and As demonstrated good recoveries (92-112%) in the FFPE tissue. The recovery of Zn and Cd were found to be in less degree, as ~40% and ~60%, respectively. The resulting data are shown in Table 3.

Table 3. Sector-field (high resolution) ICP-MS analysis of fresh and FFPE prostate tissue for Fe, Se, As, Zn and Cd. Paraffin removal from FFPE tissue was performed with xylene using tissue processor. Samples were prepared and analyzed in triplicates, the obtained concentration values are shown for dried tissue.

Sample	Tissue	Fe (µg/kg)	Zn (µg/kg)	As (µg/kg)	Se (µg/kg)	Cd (µg/kg)
1	Fresh tissue	88081 ±10929	521388 ±123510	43 ±22	1467 ±112	411 ±43
	FFPE tissue	70703 ±5676	233931 ±68542	52 ±13	1393 ±15	289 ±47
2	Fresh tissue	88317 ±5019	716115 ±390266	34 ±6	1530 ±227	422 ±47
	FFPE tissue	84965 ±37945	230905 ±51798	44 ±5	1465 ±309	289 ±71
3	Fresh tissue	108686 ±10804	407814 ±211319	25 ±1	1156 ±200	379 ±45
	FFPE tissue	84220 ±18762	326586 ±125826	32 ±9	1520 ±424	216 ±18
4	Fresh tissue	85952 ±12646	692289 ±76831	35 8±	1302 ±81	336 ±34
	FFPE tissue	98585 ±9769	282499 ±47506	35 ±2	1351 ±114	205 ±4
5	Fresh tissue	107200 ±21129	893159 ±212493	41 ±15	1576 ±91	328 ±12
	FFPE tissue	89245 ±9823	413840 ±131908	48 ±4	1537 ±121	215 ±30
6	Fresh tissue	107254 ±16244	1211934 ±102365	45 ±10	1453 ±163	271 ±4
	FFPE tissue	118086 ±15126	281401 ±61235	51 ±7	1741 ±159	182 ±24
7	Fresh tissue	104326 ±23330	854812 ±271481	73 ±15	1348 ±146	358 ±35
	FFPE tissue	84477 ±18919	155842 ±49950	50 ±7	1384 ±288	158 ±26
Recovery from FFPE tissue		92 ±16 (%)	41 ±20 (%)	112 ±21 (%)	107 ±13 (%)	62 ±9 (%)

The observed difference in the levels of each metal tested between the fresh and FFPE tissue samples originating from the same area of the prostate gland may be affected by errors in analysis, changes in the dry tissue weight occurred due to the embedding process, and/or uneven distribution of the elements within the gland (introducing a non-linear factor). This uneven distribution of each metal along the gland is directly seen from the comparison of corresponding values between different samples of the fresh tissue. Furthermore, it was previously demonstrated that concentrations of some elements can indeed vary significantly within the same gland. For example, for Zn, it was shown that its concentration may vary with up to 20-fold, and generally, increases from bladder side of the gland towards its apex and varies within the same horizontal

layers; and is high in fibromuscular stroma (23-26). In turn, Cd concentration increases from the apex side to bladder and also varies within horizontal levels of the gland with typical general difference of about 2-fold (27). Up to 2-fold difference in distribution along the gland was also demonstrated for Se (28; 29). Thus, it is likely that in our experiment the measured levels of the metals in each given portion of the tissue reflect their real content at this locus, and not significantly affected by errors during the measurements. Altogether, these data demonstrated that paraffin embedding and its removal from prostate tissue does not significantly affect the levels of Fe, Se and As, whereas Zn and Cd are subjected to certain leakage from the tissue at ~60% and ~40%, respectively. Therefore, the corresponding correction factors, 2.5 for Zn and 1.7 for Cd, should be applied to the determined levels of these metals in FFPE-tissue to assess their original content in the fresh tissue.

The developed technique allows determining Fe, Zn, Se, As and Cd in FFPE prostate tissue with high sensitivity and precision, sufficient to analyze physiological range of concentrations of these elements. With corresponding corrections for the loss of Zn and Cd during the processing, the levels of these elements in the tissue can be assessed, or alternatively, the relative content of Zn and Cd between different samples can be also informative. The technique can be useful in various biomedical studies for analysis of archival FFPE tissue, especially with well described patient's history to derive correlations between development of cancer and other diseases with exposure to above elements. The methodology developed here provides a basis for fulfilling the next objective of the project, i. e. analysis of Zn, Se and Cd (and in addition, Fe and As) in FFPE prostate tissue of patients with different relation to cancerogenesis. A manuscript describing the results obtained during the first stage of this research project is under preparation.

Key research accomplishments.

On this investigation, we have developed method for simultaneous analysis of Zn, Cd, Se, As and Fe in FFPE prostate tissue using sector-field (high-resolution) ICP-MS. We have shown that in comparison with fresh tissue, Fe, Se and As are retained in FFPE-tissue about at the original levels, whereas Zn and Cd levels were reduced at a rate of ~40% and ~60%, respectively. Thus, correction factors, 2.5 for Zn and 1.7 for Cd,

should be applied to the determined levels of these metals in FFPE-tissue to assess their original content in the fresh tissue.

Reportable outcomes.

Abstracts, presentations and a publication:

1. Todorov, T., Gray, M. A., Sarafanov A.G., Kadjacsy-Balla, A., and Centeno, J. A. Comparison between the cadmium, zinc, selenium, iron and arsenic content in fresh and paraffin embedded tissue specimen. European Conference on Plasma Chemistry, Feb 18-23, 2007, Taormina, Italy.
2. Todorov, T., Gray, M. A., Kadjacsy-Balla, A., Mullick, F. G., and Centeno, J. A. Cadmium, zinc, selenium and arsenic in fresh and paraffin embedded prostate tissues, pp 143, Abstract #O-103. 9th International Symposium on Metal Ions in Biology and Medicine, May 21-24, 2006, Lisboa, Portugal.
3. Gray, M. A., Centeno, J. A., Todorov, T., Slaney, D. P., and Nacey, J. N. Environmental exposure to cadmium, zinc and selenium and risk of prostate cancer, pp 61, Abstract #O-21. 9th International Symposium on Metal Ions in Biology and Medicine, May 21-24, 2006, Lisboa, Portugal.
4. Gray, M. A., Centeno, J. A., Slaney, D. P., Ejnik, J. W., Todorov, T., and Nacey, J. N. (2005) Environmental Exposure to Trace Elements and Prostate Cancer in Three New Zealand Ethnic Groups, *Int. J Environ. Res. Public Health* 2, 374-384.

Conclusion:

In this investigation we have developed a method for the accurate and precise determination of Zn, Cd, Se, As, and Fe in formalin-fixed paraffin-embedded prostate tissue. Our studies demonstrated that in comparison with fresh tissue, Fe, Se and As remain in FFPE tissue at about original levels. In turn, Zn and Cd remain in this tissue at ~40% and ~60%, respectively that requires applying of corrections factors 2.5 for Zn, and 1.7 for Cd to assess their original content in the fresh tissue. The technique developed here provides a basis for fulfilling the next objective of the project, the analysis of the above elements in FFPE prostate (archival) tissue of patients with different relation to cancerogenesis. The developed technique can be also useful in various studies for analysis of FFPE archival tissue for levels of Fe, Se, Zn, As and Cd.

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APPENDICES

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Comparison between the Cadmium, Zinc, Selenium, Iron and Arsenic Content in Fresh and Paraffin Embedded Tissue Specimen

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Prostate cancer is a major public health issue worldwide. There is evidence that prostate tissue Cd levels are markedly elevated in patients with prostate cancer and that Cd overload is present in the vast majority of patients. There is strong evidence that the loss of a unique capability to retain high levels of Zn is an important factor in the development and progression of malignant prostate cells. Research has found a distinct biological antagonistic effect between Zn and Cd in the human prostate gland and a decrease in Zn levels in prostate cancer. Increased Fe levels can enhance cancer cell invasion. On the other hand, studies have shown that men with higher levels of blood Se have decreased chance of developing prostate cancer.

The aim of this study is to compare levels of Cd, Zn, Se and As in both paraffin embedded tissues and frozen tissue to provide validity for the use of archived paraffin embedded materials for trace element research. For the study we used liver and kidney tissues for the initial method validation followed by prostate tissues. The prostate tissue samples were obtained from the Cooperative Prostate Cancer Tissue Resource prostate tissue bank. A non-cancerous section of the tissues was divided in two parts: (1) the first part was digested and analyzed for Cd, Zn, Se and As and (2) the second part was fixed and embedded in a paraffin block after which tissue was retrieved from the block, digested and analyzed for Cd, Zn, Se, Fe and As. The quantitative analysis was performed using a high resolution inductively coupled plasma mass spectrometer. We will discuss the reliability of Cd, Zn, Se, Fe and As in paraffin embedded tissues for both liver and kidney specimens as well as for prostate tissues. Results show good correlation for Fe, Se, Zn and Cd in the various types of tissues under investigation. Therefore, this study validates the use of paraffin embedded repository materials for research into the environmental etiology of prostate cancer and other diseases.

CADMIUM, ZINC, SELENIUM AND ARSENIC CONTENT IN FRESH AND
PARAFFIN EMBEDDED PROSTATE TISSUES

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Background: Prostate cancer is a major public health issue worldwide. Environmental factors, including trace metals have been associated with prostate cancer. Exposure to cadmium (Cd) and decrease in zinc (Zn) levels have been correlated with increased possibility of prostate cancer. Selenium (Se) has also been chosen as part of this study because of its cancer preventative properties. The AFIP has a large number of paraffin-embedded prostate repository tissues that could be utilized for prostate cancer studies; however, it is unclear if paraffin embedding influences trace element levels in tissues.

Aims: The aim of this study is to compare levels of Cd, Zn, Se and As in both paraffin embedded tissues and frozen tissue to provide validity for the use of archived paraffin embedded materials for trace element research.

Methods: For the study we used prostate tissues from Cooperative Prostate Cancer Tissue Resource prostate tissue bank. A non-cancerous section of the tissues was divided in two parts: (1) the first part was digested and analyzed for Cd, Zn, Se and As and (2) the second part was fixed and embedded in a paraffin block after which tissue was retrieved from the block, digested and analyzed for Cd, Zn, Se and As. The analysis was performed using a high resolution inductively coupled plasma mass spectrometer.

Results: In this study, we report the comparative analysis of fresh and paraffin embedded prostate tissues for Cd, Zn, Se and As. We will discuss the reliability of Cd, Zn, Se and As in paraffin embedded tissues.

Conclusion: This study validates the use of paraffin embedded repository materials for research into the environmental etiology of prostate cancer and other diseases.

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ENVIRONMENTAL EXPOSURE TO CADMIUM, ZINC AND SELENIUM AND
RISK OF PROSTATE CANCER

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Background: Our study examined putative environmental trace elements for prostate cancer; cadmium (Cd), zinc (Zn) and selenium (Se). Prostate cancer is the most common cancer diagnosed and the third most common cause of cancer deaths in New Zealand (NZ) men; with a significant difference by ethnic group. Through common occurrence (Cd) or deficit (Se, Zn), Cd, Zn and Se are significant elements within the NZ environment. The interface of ethnicity and environmental trace elements and their role in causing different rates of prostate cancer is still unknown. Cd may be a risk factor, whilst Zn and Se may interact with Cd as promising protective agents.

Aim: To explore the role of trace elements in prostate cancer.

Methods: Fourteen hundred subjects were enrolled in a cross-sectional study, tested for serum Prostate Specific Antigen (PSA) and asked to complete a comprehensive questionnaire. Within this group, we randomly selected (stratified by ethnicity) 561 subjects and measured blood Cd, Zn and Se levels using Inductively Coupled Plasma Mass Spectrometry. These trace element levels were compared with clinical, demographic, occupational and dietary data.

Results: Data showed differences in exposure to Cd, Zn and Se between ethnic groups and a positive association between Cd and PSA.

Conclusions: This positive association may indicate that Cd increases the risk of prostate cancer, and may also need to be considered when interpreting clinical PSA results.

Environmental Exposure to Trace Elements and Prostate Cancer in Three New Zealand Ethnic Groups

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Abstract: A stratified random sample of 176 men was taken from a larger community prostate study group of 1405 eligible subjects from three ethnic groups in the Wellington region of New Zealand, in order to examine ethnic differences in exposure to cadmium (Cd), selenium (Se) and zinc (Zn) and possible associations of blood levels of Cd, Se and Zn with the prevalence of elevated serum Prostate Specific Antigen (PSA); a marker of prostate cancer. Maori and Pacific Islands men were found likely to have higher Cd exposure than New Zealand Europeans through diet, occupation and smoking. However, there was no significant difference between ethnic groups in mean blood Cd levels. Pacific Islands men had significantly higher levels of blood Se than both New Zealand European men and Maori men. Maori men had significantly higher levels of blood Zn than both New Zealand European men and Pacific Islands men. A positive association was found between blood Cd and total serum PSA. Se and Zn levels were not associated with elevated PSA. Maori and Pacific Islands men have higher prostate cancer mortality rates than New Zealand European men. Ethnic differences in mortality could be contributed to by differences in rates of disease progression, influenced by exposure and/or deficiency to trace elements. However, results did not reflect a consistent ethnic trend and highlight the complexity of the risk/protective mechanisms conferred by exposure factors. Further research is needed to ascertain whether the associations found between Cd and PSA levels are biologically important or are merely factors to be considered when interpreting PSA results clinically.

Keywords: Cadmium, selenium, zinc, serum prostate specific antigen, prostate cancer, ethnicity.

Introduction

Prostate Cancer and Environmental Trace Elements

It is well accepted that genetic variation alone does not explain the observed differences in incidence of prostate cancer [1]. A 120-fold difference in rates of prostate cancer among different countries indicates that there is substantial variation in occurrence of this disease, and suggests that environmental factors are of importance [2]. The Health Research Council of New Zealand [3] highlights that the role of putative environmental and dietary factors (such as low soil Se) in causing prostate cancer is still unknown. Whether such environmental

exposures are associated with increased rates or progression of prostate cancer remains to be explored.

Within the New Zealand population there is significant ethnic variation in cancer incidence and mortality [4]. WHO age-standardized prostate cancer rates in 1998-1999, showed that at 86.1 per 100,000, Maori males had the lowest incidence, followed by Pacific Islands males at 115.2 per 100,000 and other (chiefly New Zealand European) males the highest incidence at 118.9 per 100,000 [5]. Conversely, the prostate cancer WHO age-standardized mortality rates for Pacific Islands males and Maori males in 1998-1999 (52.3 and 39.3 per 100,000 respectively) were higher than rates for other males (22.8 per 100,000) [5,6].

The ethnic disparity between incidence and mortality is likely to reflect reduced health care utilisation by Maori and Pacific Islands men, as well as underreporting in ethnic health data collection in New Zealand [7-9]. Recent research has shown that prostate cancer incidence for Maori and Pacific Islands men is likely to be at least as high as that shown for New Zealand European men [10]. The higher mortality rates shown for Pacific Islands and Maori men may reflect the health utilisation issues of later diagnosis and treatment, as well as ethnic differences in disease progression, influenced by exposure to risk factors.

Ecological and migrant studies world-wide have implicated environmental factors as risks for prostate cancer [11]. Since many environmental factors are modifiable, they lead to important implications for the prevention of prostate cancer. Three environmental trace elements, cadmium (Cd), selenium (Se) and zinc (Zn) have been highlighted in the literature in relation to prostate disease [12, 13] and are important elements within the New Zealand environment [14, 15].

Cd is readily found in New Zealand soil [16, 17]. Certain foods in New Zealand are high in Cd, including watercress, shellfish and offal meats [15]. Zn is a nutrient element found in high concentrations in the prostate gland and is necessary for healthy prostate function [12]. Cd and Zn compete for binding to protein ligands in tissues and Zn antagonises the toxicity of Cd [18]. Both Cd and Zn may affect the amount of both testosterone and dihydrotestosterone (DHT) in the prostate, which are essential for the progression of both benign prostate hyperplasia (BPH) and prostate cancer [19, 20].

The role of Se as a promising anti-carcinogen for prostate cancer is being researched internationally in clinical trials using dietary supplements [12]. Dietary intakes of Se in New Zealand are low by international comparisons [14]. The New Zealand specific characteristics of these three trace elements may mean that New Zealand males are particularly susceptible to environmentally induced prostate disease. Furthermore, there are known ethnic differences in diet, occupation and smoking levels, which may affect exposure to these trace elements.

Blood Cd reflects both recent and cumulative exposures [21]. Therefore, the older the person the higher the blood Cd level is likely to be. It has also been suggested that, as populations age, their intake of Se and Zn decreases due to age-related changes in diet [22]. As prostate cancer risk increases with age, these changes in trace element exposure may further increase prostate cancer risk.

Risk Factor - Cadmium

The human body burden of Cd has increased over the last 100 years due to an increase in environmental and industrial pollutants [15]. Environmental Cd pollution occurs in New Zealand through a combination of land contamination (through fertilisers and sludge application) and water contamination (through irrigation and industry), and is subsequently introduced into the food chain. In New Zealand, soil Cd levels are

increasing [16, 17]. Excess consumption of lamb, kidney, alcohol, grains, and oysters can increase the body burden and industry can provide direct occupational exposure [15].

Studies have attempted to find a link between Cd exposure and prostate cancer with equivocal results. Cd exposure has been positively associated with elevated prostate cancer rates in a number of occupational and laboratory studies [13, 23-26]. Research into environmentally exposed populations to Cd, do not indicate an increased relative risk of cancer [27]. However, biological studies have found evidence of carcinogenic properties in prostatic cells [28], and that the carcinogenic effect of Cd can be hormonally mediated [13, 29].

Protective Factors - Selenium and Zinc

Se is an essential trace element found in varying concentrations in the soil. Primary sources of Se in the diet are meats, eggs, dairy products and bread. Kidney, liver, and seafood are also rich in Se. In these foods, Se occurs mostly as organic compounds [30].

Ecologic studies have established an inverse correlation between soil Se levels and prostate cancer mortality [31, 32]. The Nutritional Prevention of Cancer Study found that men taking Se supplements for five years had a 65% reduction in the incidence of prostate cancer [14]. However, another large study (the Alpha-tocopherol, Beta-Carotene Cancer Prevention Study) found no association between baseline Se and prostate cancer during nine years of follow-up [33]. Though clinical studies have focused on Se supplementation as a protective factor in reducing prostate cancer incidence [34, 35], the effect of low Se on potentially increasing prostate cancer risk has not been addressed.

A case-control study on 9345 Japanese-American men found an inverse association between blood Se levels and prostate cancer incidence [36]. This Japanese study showed that subjects had less chance of prostate cancer if baseline blood Se was greater than 147µg/L. The association was mainly present in current or past cigarette smokers [36]. This lends support to the possibility that Se may help to combat the toxic effect of cigarette compounds, such as Cd [12]. A smaller (3059 men) USA case/control study's results were consistent with this finding and reported a steady reduction in prostate cancer risk with serum Se >135µg/L [37].

Because Se concentrations in food depend on local soil conditions, intakes vary geographically. Some populations, living in parts of the world that depend on domestic food production, ingest very little Se and are at risk of Se deficiency [37]. New Zealand is low in soil Se, particularly in some areas of the South Island and this is reflected in low Se levels in some foods that are domestically grown [14].

A report by Thomson and Robinson [38] concluded that the Se status of New Zealanders had improved since the first reports in the early 1970s. This improved Se status was attributed to the importation of Australian wheat and cereal because of its higher Se content, as well as an increased consumption of fish and poultry [38].

However, both the 1997/98 New Zealand Total Diet Survey and the 1997 National Nutrition Survey found that the dietary intakes of Se were well below the recommended daily allowances in other countries [39]. Intake estimates from the 1997 National Nutrition Survey were only two-thirds the Australian recommended daily intake. More recent studies in younger New Zealand adults still indicate an insufficient Se intake for full expression of glutathione peroxidase (GPx) and do not confirm the earlier findings of 1996 [22].

Furthermore, in the South Island, 100% New Zealand grown wheat is used in bread making, whereas in the North Island, between 50 and 100% imported wheat is used [22]. As older people are prone to low Se status and because intakes of Se are strongly correlated with those of total energy and protein [40], de Jong et al. [22] conclude that this places the elderly living in the South Island of New Zealand at particular risk.

Zn is a homeostatically regulated essential mineral, present in red meat, poultry, grains, dairy, legumes and vegetables. It is a component of numerous metalloenzymes and is important for cell growth and replication, osteogenesis and immunity. Zn may also act as an antioxidant by stabilizing membranes in some cell types. Some studies have found an association of lower Zn intakes in patients with certain cancers, whilst others have observed no association [12].

Zn is regarded as a 'cellular growth protector' for the prostate. Normal human prostate accumulates the highest levels of Zn of any soft tissue in the body [41]. In contrast, the Zn level in prostate cancer cells is markedly decreased from the level detected in non-prostate tissues [42]. Studies have found evidence that Zn inhibits human prostate cancer cell growth; possibly due to induction of cell cycle arrest and apoptosis [42]. It is thought that the loss of a unique capability to retain high levels of Zn is an important factor in the development and progression of malignant prostate cells [42]. In-vitro Zn helps to maintain intra-prostatic balance of testosterone and DHT [41].

Based on these cellular activities, it could be expected that Zn levels would be inversely associated with prostate cancer. However, epidemiologic findings for Zn and prostate cancer incidence and mortality have not been consistent. At this time the evidence for a beneficial effect of Zn on prostate cancer incidence is insufficient to warrant undertaking randomized chemoprevention trials [12].

Reduction in red meat and an increase in cereals in the diet may compromise the intake and bioavailability of Zn in New Zealanders diet [43]. Both dietary and biochemical data suggest that current 'Western' diets of the elderly may induce a considerable risk of Zn deficiency. The detrimental effect of a marginal Se status on immune function may be further aggravated by inadequate Zn intake [43].

A study by Feustel et al. [44] found a distinct biological antagonistic effect between Zn and Cd in the human prostate gland. They found an increasing amount of Zn in cases of benign prostatic hyperplasia (BPH) but a decrease in prostate cancer. In contrast, they found a lower Cd concentration in the normal prostate compared

to prostate cancer. Studies in the late 1980s confirmed that elevated total PSA (>4.0ng/mL) could be used to identify patients with prostate cancer [45]. A recent study has found a relationship between Zn in prostate tissue and PSA levels in blood [46].

Ethnic differences in prostate cancer mortality could be contributed to by differences in rates of disease progression, influenced by exposure to trace elements. The aim of this study was to examine ethnic differences in exposure to Cd, Se and Zn and possible associations of blood levels of these trace elements with the prevalence of elevated PSA in three ethnic groups.

Materials and Methods

From January 2000 to February 2002, a total of 1617 males were recruited into the Wellington Regional Community Prostate Study (WRCPS) co-ordinated through the Wellington School of Medicine and Health Sciences of the University of Otago. In order to ensure an adequate representation of Maori and Pacific Islands men, participants were enrolled by two separate procedures. Initially subjects were identified in census area units containing at least 5% Maori and 5% Pacific Islands populations. Males aged between 40 and 69 years were invited to attend a local clinic where a blood sample was taken and a detailed questionnaire was completed (Phase 1). The total number recruited into Phase 1 was 698.

The second mode of recruitment was through the identification of individuals who had been screened as part of the Wellington hepatitis and diabetes-screening programme for Maori and Pacific Islands populations. After ethics approval, blood samples were retrieved from the Hepatitis Foundation of New Zealand and subjects were contacted and asked to complete the study questionnaire (Phase 2). The total number recruited into Phase 2 was 919.

A 10ml tube of blood was collected, and centrifuged within 4 hours of sampling. Serum was stored at -70C until assayed. Sera from each subject were tested for total PSA, complexed PSA (cPSA) and free PSA (fPSA). Laboratory blood analysis for PSA and free PSA was carried out using an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany) assay [47]. Assays were performed according to manufacturer's specifications.

Questionnaires were self-administered and covered occupation, education, medical history and diet. Most dietary information was obtained via a subset of questions from a food frequency questionnaire (FFQ) previously validated in another New Zealand study [48].

Ethnicity was determined on a self-identification basis [49]. Subjects also completed an International Prostate Symptom Score Sheet (IPSS) [50] and declared if they have ever had any evidence of prostate disease. Subjects with total PSA levels of >4.0ng/mL were referred back to their general practitioner for further evaluation and exclusion of prostatic malignancy.

In order to ensure subjects were affiliated to the three ethnic groups in question and aged between 40-69, age and ethnicity selection criteria were applied to the combined Phase 1 and Phase 2 group (1617). Subjects were also excluded on the basis of suspected prostate cancer (through PSA testing, GP diagnosis or prostate

biopsy). A stratified random sample was taken from the remaining 1405 eligible subjects.

Sampling was undertaken as follows; 150 randomly chosen New Zealand European, Maori and Pacific Islands men (50 from each group), plus all 26 men who screened positive for elevated PSA (total PSA >4.0ng/mL) were selected to be tested for Cd, Se and Zn. Due to some results being below the detection limits, data were only available for 157 subjects; 51 New Zealand European, 60 Maori and 46 Pacific Islands men.

Laboratory Analyses

Several methods have been used for the analysis of Zn, Cd and Se in biological samples and environmental samples including flame and graphite furnace atomic absorption (FAA and GFAA), Inductively Coupled Plasma Emission Spectrometry (ICP-OES) as well as Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [51]. ICP-MS and GFAA are most commonly used for the clinical application. The levels for Cd and Se in blood are in $\mu\text{g/L}$, whereas for Zn the levels are in mg/L (or $\mu\text{g/mL}$) of blood [52]. The detection limits for both techniques for all three elements are below these levels (low $\mu\text{g/L}$ for GFAA and ng/L for ICP-MS) [51]. However, GFAA suffers from short dynamic range and each analyte requires a separate analysis [51]. The advantages of ICP-MS are short analysis time, multi-element capability and linear response over several orders of magnitude (from low ng/L to mg/L within the same run) for each of the elements studied [51, 53]. For these reasons ICP-MS was the selected method for the current study.

Cd, Se and Zn measurements were carried out using ICP-MS at the Division of Biophysical Toxicology Pathology, U.S. Armed Forces Institute of Pathology in Washington DC [54]. We used open vessel microwave digestion (MDS 2000, CEM Corporation, Mathews, NC, USA) for the New Zealand blood samples, using internal standards for all three elements (^{74}Se , ^{68}Zn and ^{106}Cd). Finally, the samples were dissolved in concentrated nitric acid and diluted accordingly for ICP-MS measurement and analysis [54]. The analysis was performed on Perkin Elmer Sciex Elan 6100 DRC instrument (Perkin Elmer, Norwalk, CT, USA).

Certified reference materials (National Institute of Standards and Technology, Gaithersburg, MD) of the same matrix, at known concentrations, were used as quality controls and these were used at intervals of every batch of 10-15 samples per analysis. This method provided percent recoveries for each of the analytes between 95-105%.

Statistical Analyses

Statistical analyses were performed with SPSS version 10.1. Two separate sets of analyses were performed; first to examine the ethnic-specific levels of Cd, Se and Zn, second to investigate the relationship between these trace elements and PSA.

Ethnic Differences in Trace Elements

As blood levels of Cd, Se and Zn are associated with age, a standard multivariate regression model was used to

estimate the age-adjusted mean levels in $\mu\text{g/L}$ (or $\mu\text{g/mL}$ for Zn) of the trace elements for each ethnic group.

Occupational data were sorted into two categories; those who currently work in high Cd exposure risk jobs and those who do not. Linear regression analyses were undertaken to examine the relationship between the blood trace element values, diet, high Cd exposure risk occupation (such as metal work) and smoking data. Ethnic differences in consumption of foods were investigated [55]. Life in New Zealand (LINZ) Activity and Health Unit [56] results were used to assess likely sources of trace elements from food.

Zn and Se results were compared with other available New Zealand studies [22, 57, 58] and major overseas studies [36, 37, 59, 60]. No comparable studies were available for Cd.

PSA Levels and Trace Element Associations

Because Cd was significantly associated with total PSA levels, weighting by WRCPS group proportions of elevated PSA was undertaken in order to standardize for differences in the structure of elevated PSA prevalence between the sample group and the larger WRCPS group.

Cd levels were categorized according to the percentile blood Cd results of a large sample of subjects >20 years old (4207) in the US NHANES between 1999 and 2000 [21]. Three categories were developed for the New Zealand population; low = <LOD-0.60 $\mu\text{g/L}$, medium=0.60-1.00 $\mu\text{g/L}$ and high >1.00 $\mu\text{g/L}$. These Cd categories were used to determine the extent of PSA increase with increased levels of blood Cd.

Linear models were undertaken to estimate the effect of all the trace elements on total PSA, complexed PSA (cPSA), free PSA (fPSA) and the ratio of free to total PSA (%fPSA). Because PSA, cPSA, fPSA, Cd, Se and Zn were found to be log-normally distributed, the logarithm of all biomarkers was used in analyses. A logistic regression model was developed to test whether the prevalence of elevated PSA varied significantly by levels of Cd, Se and Zn.

To test for possible bias in the stratified sampling method, a comparison of p-values in regression models was undertaken after the removal of all men with elevated PSA (>4.0ng/mL). Backwards Stepwise Regression models were developed to examine if the association between trace elements, initially found to correlate to PSA (and its various molecular derivatives) on a bivariate level, was due to confounding alone [61].

Potential confounding factors for elevated PSA such as age, smoking, body mass index, lower urinary tract symptoms, selected dietary factors and occupation were controlled for within the models. Due to the non-random sampling method in the WRCPS, the potential for selection bias was investigated and partially controlled for by including factors within the model likely to be associated with participation, such as Phase of recruitment.

Results

Statistical tests for potential confounding and selection bias were undertaken. There was no change in the significance of p-values after the removal of men with

elevated PSA (>4.0ng/mL) indicating that the stratified sampling method (of including all men who had elevated PSA levels) was not likely to bias the results.

Standardization of mean blood Cd levels by weighting by population proportions of elevated PSA was undertaken. Results indicated that the actual blood Cd mean was 1.03 and weighted mean was 1.04 (with overlapping 95%CI). As the standardized rate did not differ greatly from the crude rate, the confounding effect of differences in the structure of elevated PSA prevalence between the sample group and the larger group was small [62].

Ethnic Differences in Exposure to Trace Elements

Tables 1 and 2 show that, in mean blood Cd levels, there was no significant differences between the ethnic groups. Pacific Islands men have significantly higher levels of blood Se (164.14µg/L) than both New Zealand European men ($p<0.001$, 117.87µg/L) and Maori men ($p<0.001$, 122.30µg/L). Zinc levels were significantly higher in Maori men (6.94µg/mL) than both New Zealand European ($p=0.002$, 6.38µg/mL) and Pacific Islands men ($p=0.003$, 6.36µg/mL).

Table 1: Age-adjusted mean blood level for total study group and each ethnic group.

Trace Element	Total	New Zealand European Men	Maori Men	Pacific Islands Men	
Cd µg/L	1.04 ^{*a} (0.86-.22)	1.03 (0.50-.08)	0.93 (0.79-1.08)	1.04 (0.90-1.21)	1.15 (0.98-1.36)
Se µg/L	131.72 (129.71-133.73)	117.87 (109.07-127.38)	122.30 (113.71-131.53)	164.14** (151.02-178.40)	
Zn µg/mL	6.58 (1.98-8.57)	6.38 (6.13-6.63)	6.94* (6.69-7.20)	6.36 (6.10-6.64)	
Zn/Cd Ratio	7.57 (6.82-8.31)	7.98 (6.66-9.29)	7.62 (6.41-8.84)	7.04 (5.65-8.42)	

^{*a} Mean Cd standardized for prevalence of elevated PSA. 95% CI are shown in brackets.

Table 2: Difference in blood trace element mean level between the three ethnic groups (p-value)

Trace Element	New Zealand European vs Maori	New Zealand European vs Pacific Islands	Maori vs Pacific Islands
Cd	0.261	0.055	0.363
Se	0.495	<0.001**	<0.001**
Zn	0.002*	0.956	0.003*
Zn/Cd ratio	0.699	0.334	0.932

All least significant differences are reported and significant differences (p-values) determined using the Holm multiple comparison procedure [$p1 = 0.05 / (n-i+1)$].

Table 3 shows that New Zealand European and Maori men had blood Se levels lower than those shown for the three ethnic groups in the USA (all between 132-140µg/L) [36, 37]. Pacific Islands men have comparable blood Se levels (within the 95%CI) to the highest shown for American Europeans (140µg/L, 95%CI 85-195µg/L) [37]. Results were significantly higher than found in a Finnish population in 1990 (62.2µg/L, 95%CI 33.1-91.3µg/L) [60]. Mean Se results were generally higher than other New Zealand studies. However, results for New Zealand Europeans and Maori were within the 95%CI of the results reported for Otago women (114µg/L, 95%CI 66-162µg/L) and Otago women > 70 years old (97µg/L, 95%CI 49-145µg/L) [22]. Pacific Islands men's mean blood Se results were significantly higher than results reported for Otago women >70 years old.

Table 4 shows that all three ethnic groups had blood Zn levels significantly lower than those shown for the US NHANES (8.56µg/L, 95%CI 8.55-8.57µg/L) [56]. All three ethnic groups were comparable to a Spanish study, which found adjusted mean blood Zn levels to be 6.95µg/mL (95%CI 5.87-8.03µg/mL) [63]. Mean results were shown to be generally higher than other New Zealand studies, although total mean blood Zn levels (6.58µg/mL, 95%CI 1.98-8.87µg/mL) were within the 95%CI (1.05-1.65µg/mL) of the study on Otago women [43]. Some Se and Zn study comparisons are not based on analyses of the same blood derivatives therefore may be indicative of differences rather than precise comparisons (see tables for study blood derivative details).

Table 3: Blood Se level by ethnicity of the WRCPS, compared with other community-based studies.

Study	WRCPS ^{c*}			Robinson et al. [57] ^{c*}	de Jong et al. [22] ^{d*}	Voght et al. [37] ^{e*}	Nomura et al. [36] ^{e*}	Knekt et al. [60] ^{e*}			
	All	New Zealand European	Maori	Pacific Islands	New Zealand (Otago)	New Zealand (Otago Women)	New Zealand (Otago Women >70 yrs)	African American	American European	Japanese American	Finland
n	157	51	60	46	146	Not published	103	112	121	9345	39,268
Se µg/L	132 ^{a*}	118 ^{a*}	122 ^{a*}	164 ^{a*}	54	114 ^{b*}	97 ^{b*}	134	140	132	62.2

^{a*} These are age-adjusted values.

^{b*} Values have been converted from µmol/L to µg/L by the formula µg/L = (µmol/L x 1000)/8.90

^{c*} Se levels have been determined on whole blood. ^{d*} Se levels have been determined on plasma.

^{e*} Se levels have been determined on serum.

Table 4: Blood Zn level by ethnicity of the WRCPS, compared with other community-based studies.

Study	WRCPS ^{c*}			McKenzie [58] ^{d*}	Gibson et al. [43] ^{d*}	de Jong et al. [22] ^{d*}	Hotz et al. [59] ^{d*}	Moreno et al. [63] ^{c*}	
	All	New Zealand European	Maori	Pacific Islands	New Zealand (Otago Women)	New Zealand (Otago Women >70 yrs)	New Zealand (Otago Women >70yrs)	USA NHANES	Spanish
n	157	51	60	46	49	330	103	14,770	82
Zn µg/mL	6.58 ^{a*}	6.38 ^{a*}	6.94 ^{a*}	6.36 ^{a*}	2.09	1.35	1.39	8.56	6.95

^{a*} These are age-adjusted values.

^{b*} values have been converted from µmol/L to µg/mL by the formula µg/L = µmol/L/8.90

^{c*} Zn levels have been determined on whole blood.

^{d*} Zn levels have been determined on serum.

Exposures - Diet, Smoking and Occupation

Ethnic differences in consumption of foods were investigated [55]. The most significant difference in dietary practice was between New Zealand European and Pacific Islands men. The age group studied (40-69 years) probably influenced the magnitude of this difference. Other studies have shown that this generation of Pacific Islands people have traditional diets, eating more taro, shellfish and fresh vegetables, and drinking less alcohol than New Zealand Europeans [64]. The dietary pattern of Maori found in the WRCPS more closely resembled that of New Zealand European men, which may be because Maori now live a more 'Westernised' rather than traditional lifestyle [65].

Blood Cd was significantly positively associated with shellfish intake ($p=0.039$) when adjusting for smoking status. As discussed later in this section, Pacific Islands and Maori men have significantly higher intakes of shellfish than New Zealand European men.

More Maori and Pacific Islands men (31% and 31% respectively) were current smokers than New Zealand European men (17%). Current smokers had significantly higher blood Cd levels ($1.77\mu\text{g/L}$, 95%CI 1.58-1.96 $\mu\text{g/L}$) than former smokers ($p<0.001$, $0.977\mu\text{g/L}$, 95%CI 0.817-1.14 $\mu\text{g/L}$) and never smokers ($p<0.001$, $0.886\mu\text{g/L}$, 95%CI 0.707-1.07 $\mu\text{g/L}$).

More (39.4%) Pacific Islands men currently work in occupations deemed within the literature to be high Cd exposure risk than New Zealand European (17.6%) and Maori men (20%). 83.4% of those who currently worked in high Cd exposure risk occupations had been in this work for more than 2 years. For these men, the average amount of time spent working in these occupations was 17 years. However, blood Cd levels were not associated with working in a high Cd exposure risk occupation ($p=0.161$).

Pacific Islands males had significantly higher intakes of combined fish/shellfish than Maori and New Zealand European men ($p<0.001$), and Maori higher intakes of combined fish/shellfish than New Zealand European ($p<0.001$). Pacific Islands and Maori males had significantly higher intakes of pork, lamb and vegetables than New Zealand Europeans ($p<0.001$).

PSA Levels and Trace Element Associations

A positive association was found between blood Cd and total PSA ($p<0.001$, $r=0.226$), which remained significant within the multivariate model. This association also remained significant after removal of men with elevated PSA.

A positive association was found between being in the elevated PSA group and blood Cd levels. For example, having a blood Cd level $<1.00\mu\text{g/L}$ was associated with an 83% reduction in elevated PSA risk (OR 0.17, 95%CI 0-1.68, $p=0.024$). No association was found between Se and Zn levels and elevated PSA.

The mean PSA level in the low blood Cd level category was 0.85ng/mL (95%CI 0.12-1.33 ng/mL), medium category was 0.90ng/mL (95%CI 0.09-1.23 ng/mL) and high blood Cd level category was 1.64ng/mL (95%CI 0.40-2.52 ng/mL). There was a

significant difference in PSA levels between the low and high blood Cd level ($p<0.001$).

Discussion

Ethnic Differences in Exposure to Trace Elements

There was no significant difference in blood Cd levels between the three ethnic groups. However, a difference in Cd levels was expected, as Maori and Pacific Islands men both have higher exposure to factors significantly associated with increased blood Cd levels (such as diet, occupation and smoking).

Shellfish in New Zealand are high in Cd [15], and our data indicated that shellfish intake was significantly higher in Maori and Pacific Islands men than New Zealand European men. Blood Cd was found to be significantly associated with shellfish intake. In addition, more (39.4%) Pacific Islands men currently work in high Cd exposure risk occupations, and more Maori and Pacific Islands men are current smokers (both 31%). Nevertheless, these factors did not result in a significant difference in blood Cd levels between the three ethnic groups.

Luoma et al. [66] found that the Cd concentration of smokers was three times that of non-smokers ($1.85\mu\text{g/L}$ vs. $5.5\mu\text{g/L}$). Even though differences in blood Cd according to smoking status were smaller than found in Luoma et al.'s [66] study, our results showed that current smokers (at $1.77\mu\text{g/L}$) had 1.9 times the blood Cd level of former smokers ($0.977\mu\text{g/L}$) and twice the level of never smokers ($0.886\mu\text{g/L}$).

It is possible that ethnic difference in Cd did not reach a significant level because New Zealand European men are exposed to other sources of Cd (not accounted for within this research), and/or because there is an ethnic difference in metabolizing this metal and/or interactions between trace elements.

Whether blood Cd at the levels reported in this study is a cause for health concern is not known. Finding a measurable amount of Cd in the blood does not automatically lead to an adverse health effect. Occupational Safety and Health organisations worldwide, have developed (or adopted) criteria for evaluating occupational chronic workplace exposures. In the USA the blood Cd criterion is $>5.00\mu\text{g/L}$ [21]. This cut-off provides an upper occupational limit in which to assess this study group from. There were no men in this study whose blood Cd level reached this limit, with the 95th percentile being $2.42\mu\text{g/L}$.

A Belgian study tested blood Cd levels from both low and high environmental exposure areas [67]. Cd levels found in blood ($0.96\mu\text{g/L}$ vs. $1.24\mu\text{g/L}$ respectively) were significantly ($p<0.001$) raised in the two high exposure areas compared with the two low exposure areas ($p<0.001$) [67]. This indicates that, despite levels in this study not being high enough to be deemed chronic occupational exposure, there may be higher environmental exposure in the Pacific Islands group at $1.15\mu\text{g/L}$ (0.98-1.36) (Table 1).

There is no reason to suspect that environmental Cd is significantly higher in the Wellington region than any other large urban area in New Zealand, therefore the data

presented here provide a reference range that could help to determine whether people are exposed to higher levels of Cd than what could be considered normal for New Zealand.

Data showed that Pacific Islands men have significantly higher levels of blood Se than both New Zealand European men and Maori men (Tables 1 & 2). Tinned fish was positively associated with blood Se levels. The LINZ Activity and Health Unit (1999) reported that the top contributor to Se intake in 45-74 year old New Zealand males is fish/seafood (32%). As intake of tinned fish was shown to be significantly higher in Pacific Islands men, it is possible that their significantly higher blood Se levels were contributed to by their high consumption of tinned fish, as well as other seafood.

It is acknowledged that there are limitations in directly comparing this current study with the trace element results of other studies, because of the previously discussed possibility of different Se and Zn intakes due to geographical location and/or age. Women may also have different intakes of Se and Zn than men. Regardless, comparisons provide an important indication of trace element status in New Zealand.

With regards to Se, New Zealand European and Maori men had blood Se levels lower than those shown for the three ethnic groups in the USA (Table 3) [36, 37]. However, results were higher than found in a Finnish population in 1990 [60]. In comparison with other countries, Finland is known to be a low Se region, likely due to geological conditions [68].

Mean Se results for this current study were generally higher than other New Zealand studies compared, especially for Pacific Islands men. Pacific Islands men's mean blood Se results were significantly higher than results reported for Otago women >70 years old and Pacific Islands men have comparable blood Se levels to the highest shown for American Europeans (Table 3) [37].

A comparison of the 1997 New Zealand National Nutrition Survey [56] with the US 1988-1994 NHANES found that the dietary intake of Zn for the New Zealand population was higher than that for the US, however, seniors (>71 years old) in both surveys had the lowest Zn intakes [69].

Mean Zn results for this current study were generally higher than the other New Zealand studies compared. Data show that all three ethnic groups had blood Zn levels comparable to a Spanish study [63] and, contrary to the findings of the dietary intake estimate study [69], significantly lower than those shown for the US NHANES (Table 4) [59].

Zn levels were significantly higher in Maori men than both New Zealand European and Pacific Islands men (Tables 1 & 2). This may in part be due to their higher intake of pork, lamb and vegetables, which are the next highest contributors of Zn (approx. 15% total) after beef, bread and milk for 45-74 year old New Zealand males [56]. Despite these differences in blood Zn levels, it appears that blood Zn levels found in this current study, for all ethnic groups, are suggestive of adequate Zn levels for general health. For example, Pilch and Senti [70] suggest that serum levels <10.7 μmol/L (or

1.2 μg/mL) indicate Zn deficiency. No men in this study were below this level (Table 4), however, it is unclear if the levels reported in this study are adequate for prostate health.

PSA Levels and Trace Element Associations

In New Zealand, more Pacific Islands and Maori men die from prostate cancer than New Zealand European men [5]. It is presently unknown whether the ethnic difference in mortality is influenced by exposure to trace elements. This aspect of the study aimed to explore whether ethnic differences in blood levels of Cd, Se and Zn and a marker of prostate cancer, PSA, might explain differences in prostate cancer mortality.

In our study, a positive association was found between blood Cd and total PSA, and an association was found between having an elevated PSA and increased blood Cd levels. The effect of Cd on PSA levels could be due to direct prostate cellular changes towards cancer [13, 29] or via hormonal pathways involving androgens. PSA expression is differentially regulated by androgens acting on prostate cells [45] and androgenic activity has been shown to be influenced by Cd [71]. For example, a study that treated human prostate cancer cells with Cd stimulated cell growth, found that Cd decreased the concentration of the androgen receptor protein and mRNA (80 and 60% respectively) and increased the expression of PSA (6-fold). Cd blocked the binding of androgen to the receptor but did not alter its affinity, suggesting that the metal is an inhibitor of hormone binding [71].

Regardless of the mechanism, if elevated PSA indicates higher risk for prostate cancer, the increased odds of being in the elevated PSA group with higher levels of blood Cd could indicate that Cd exposure is a risk factor for prostate cancer. This finding highlights that the potentially high level of Cd exposure of New Zealand men, in general, may be reflected in the high rate of prostate cancer in New Zealand by world standards. Ethnic differences in Cd exposure may influence New Zealand ethnic differences in prostate cancer rates.

Despite other studies describing a potential protective relationship between Se and Zn and prostate cancer [31, 32, 37, 72], this current study found no association between blood Se levels and PSA. Perhaps, Se and Zn protect prostate cells against cancer, without influencing the expression of PSA in normal prostate tissue, because these trace elements have a direct cellular (rather than humoral) effect.

It is possible that an ethnic difference in genetics influences the toxicology of trace elements and in turn, the degree of protection offered by these trace elements. For example, a study that compared prostate cancer tissue from African American men with American European men (for the ability to express two major human Zn transporters, hZIP1 and hZIP2) found that the degree of Zn expression was higher in the American European men than the African men. The study also found a significant downregulation of Zn transporters in normal prostate tissues from African American men [73]. Rishi et al. [73] suggest that because Africa is

mineral-rich and Zn levels in the water and diets are high, Africans have genetically downgraded Zn absorption capacity in order to avoid possible Zn toxicity. Furthermore, important interactions of Se and Zn with other trace elements, such as Cd, may influence prostate cancer rates, as with Wasowicz et al. [74], who found an inverse linear correlation between blood Se and Cd concentrations in workers exposed to Cd ($p < 0.001$; $r = -0.449$).

In New Zealand ethnic prostate cancer incidence rates are inaccurate because of under-reporting in health data for Maori and Pacific Islands men. There are ethnic differences in mortality rates, with Pacific Islands and Maori men shown to have higher rates than New Zealand Europeans [6]. Under-utilisation of the health system is a likely influence in the increased mortality rates for Pacific Islands and Maori males [7]; however, ethnic differences in disease progression may also contribute. Given the probable inaccuracy of the incidence rates, it appears important to examine mortality as an indication of increased disease burden for Pacific Islands and Maori men.

A large proportion of Pacific Islands men blood Se levels were over the level reported to be protective for prostate cancer. Even though Maori men had a high blood Zn status, no studies have indicated a threshold blood Zn level that may be protective of prostate cancer. This high Se and Zn status in Pacific Islands men and Maori men respectively appears to be inconsistent with their higher mortality rates in comparison to New Zealand Europeans.

The strength of this study was the recruitment of a large number of Maori and Pacific Islands men, allowing for insight to be gained into differing dietary and occupational patterns, and blood levels of Cd, Se and Zn. Standardization of mean blood Cd levels resulted in similar mean levels (Table 1). Therefore, results gained through the stratified random sample were not expected to differ markedly from those from a simple random sample. There were limitations, including the potential inaccuracy involved with dietary assessment. In this case, through the brief FFQ which did not cover total dietary intake; thus, it was not possible to control for other dietary factors. However, it is reassuring that the dietary patterns found in this study were generally consistent with those found in the 1997 New Zealand National Nutrition Survey [64]. Furthermore, there was consistency between the blood results and dietary intake estimates, which provides reassurance about the validity of blood Se and Zn measures as an indication of regular intakes of the trace elements.

It is acknowledged that Se consumed in food exists in a number of different organic and inorganic forms including selenomethionine, selenocysteine, selenate and selenite. Studies have established that the bioavailability and body distribution of Se depends upon the chemical form. For example, selenomethionine is retained in tissue proteins to a greater extent than selenocysteine and other inorganic forms. A number of other factors may influence Se bioavailability, including other dietary components; therefore, Se speciation along with the measurement of GPx activity is important considerations in further research [38].

Conclusions

Ethnic differences in mortality could be contributed to by differences in rates of disease progression,

influenced by exposure to the trace elements examined. The discovery of a significant ethnic difference in levels in Se and Zn warrants further investigation. However, results did not reflect a consistent ethnic trend and highlight the complexity of the risk/protective mechanisms conferred by exposure factors. The body burden of Cd is likely to increase with age and the intake of Se and Zn decrease. As prostate cancer risk increases as men get older, these age-related changes in trace element exposure may be an important factor to consider in prostate cancer risk. Further research is needed to ascertain whether the associations found between Cd and PSA levels are biologically important or are merely factors to be considered when interpreting PSA results clinically.

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