CHAPTER III
RESULTS

Characteristics of the subjects

Two hundred and twenty four people living in the polluted area of Mae Sot were included in this study. They comprised 104 men and 120 women. All of them were older than 40 years old. No participant had occupational exposured to Cd. The average age, urinary Cd and duration of residence were not significantly different between the 2 groups (control and experimented). Table 1 shows the mean age, weight, height, and body mass index of the subjects including occupational history, present health status, diseases history, smoking and dietary habit, and alcohol consumption. Ninety eight percent of the subjects were born in Tak. Ninety two percent of them were farmers, being the most common occupation among the people in this area. Most of the farmers (96%) ate household rice, which was grown in their own paddies. Water sources for drinking were well water (84%), rain water (72%), bottled water (20%), tap water (14%) and river water (4%). The proportion of smokers including current smokers and ex-smokers was higher in men (85%) than in women (44%). Most common diseases in the subjects were hypertension (26%) and anemia (23%). The other diseases were urinary tract stone (9%), renal or urinary tract disease (7%), osteoporosis (6%), diabetes (5%) and bone fracture (2%), as summarized in Table 1.

Qualitative analysis

All 224 participants, both men and women, had no apparent clinical symptoms related to Cd toxicity. However, when using urinary strips for on-site screening examination, 75 people (men = 41, women = 34) had positive urinary proteins. The positive rate of protein in the urine, shown by the color on the strips, ranged from yellow (negative) through yellow-green and green to green-blue for positive reactions, depending on the concentrations of proteins in the urine. Eight people (men = 2, women = 6) had positive urinary glucose. Positive glucose in the urine showed a color on the strip that ranged from green to brown. None of the participants showed an abnormal level of urinary blood, ketone body or urobilinogen. Seventy-one people (men = 41, women = 30) had a urinary pH equal to or lower than 5 (orange color). In these low pH urinary samples, one drop of 0.5N sodium hydroxide was added to adjust the pH of the urine to 6 – 8 in order to preserve β_2-MG in the urine for quantitative analysis.

Table 2 shows the positive rates of all tests. Urinary proteins usually represent an abnormality of the glomerular filtration barrier. The test is more sensitive for albumin than other proteins. Due to the negative charge of albumin, if protein (albumin) is presented in the urine, the pH increases, and the protein strip test is shown as positive. The sensitivity of albumin on the test is between 15-30 mg/dl. The positive rate is separated into 6 categories; none or normal, trace positive, positive for albumin at concentrations of 30, 100, 300, and 1,000 mg/dl. In general, the presence of glucose in urine indicates that its filtered load exceeds its in maximal tubular reabsorptive capacity. The sensitivity of the glucose test is having glucose between 75-125 mg/dl. The positive rate is separated into 6 categories; negative or normal and trace positive in glucose concentrations of 0.1, 0.25, 0.5, 1 and 2 mg/dl. The occult blood test is positive in the presence of red blood cells or free hemoglobin. Hemoglobin is usually bound to proteins and it will be too large to pass through the glomerular filter. If the renal threshold is exceeded, the hemoglobin can pass into the urine. The presence of free red blood cells results in a positive test when the blood cells lyse and hemoglobin is released. Healthy people should have negative test results. The blood test is sensitive when the hemoglobin is presented in concentrations of between 0.015-0.045 mg/dl. The positive rate is separated into 5 categories; negative or normal, trace positive, positive one plus, 2 plus and 3 plus. Urine ketone body testing only detects acetoacetic acid, and not the other ketones, acetone or beta-hydroxybuteric acid. The sensitivity of the ketone body is 5-10 mg/dl. The positive rate is separated into 6 categories; negative or normal and trace positive in concentrations of 5, 15, 40, 80 and 160 mg/dl. Urine urobilinogen is increased in any condition that causes a growth in production or retention of bilirubin, and sensitivity occurs with bilirubin concentrations of 0.1-10.0 unit /dl. The positive rate is also separated into 6 categories; negative or none and normal or trace positive with bilirubin concentrations of 0.1, 1, 2, 4, and 8 unit/dl. The pH of urine varies depending on its acid-base status. The pH in urine is divided into 7 categories; 5, 6, 6.5, 7, 7.5, 8 and 8.5. The sensitivity of the pH is 5-8.5.

In this study, distribution of the subjects with positive urinary protein is shown in Table 3. The total rate of positive urinary protein subjects including +/- results was 33.48%. The rate of positive urinary protein, classified by gender, was 39.42% in men and 28.33% in women.

Table 1 Number of subjects in the study area classified by demographic and health data

Characteristic/health information	Total (n=224)	Men (n=104)	Women (n=120)
Age (years)	57.36 ± 11.1[a]	58.3 ± 12.5[a]	56.5 ± 9.7[a]
Weight (kg)	52.54 ± 10.08[a]	52.59 ± 10.04[a]	52.66 ± 10.12[a]
Height (cm)	156.68 ± 7.70[a]	166.11 ± 7.72[a]	156.13 ± 7.73[a]
Body mass index (kg/m^2)	21.6 ± 0.17	20.61 ± 0.17	21.64 ± 0.17
Duration of residence (years)	56.28	56.02	56.54
Drink tap water (n)	31 (14%)	15 (14%)	16 (13%)
Drink rain water (n)	162 (72%)	76 (73%)	86 (72%)
Drink well water (n)	189 (84%)	88 (85%)	101 (84%)
Drink river water (n)	9 (4%)	2 (2%)	7 (6%)
Drink bottled water (n)	46 (20%)	18 (17%)	28 (23%)
Current smokers (n)	101 (45%)	67 (64%)	34 (28%)
Ex-smokers (n)	41 (18%)	22 (21%)	19 (16%)
Non-smokers (n)	82 (37%)	15 (15%)	67 (56%)
Drink alcohol (n)	109 (49%)	87 (84%)	22 (18%)
Hypertension (n)	59 (26%)	27 (26%)	32 (27%)
Anemia (n)	51 (23%)	19 (18%)	32 (27%)
Urinary tract stone (n)	20 (9%)	13 (12%)	7 (6%)
Renal or urinary tract disease (n)	15 (7%)	12 (11%)	3 (2%)
Osteoporosis (n)	13 (6%)	2 (2%)	11 (9%)
Diabetes (n)	12 (5%)	7 (7%)	5 (4%)
Bone fracture (n)	5 (2%)	4 (4%)	1 (1%)

[a]: mean ± S.D.
n: number of subjects

Table 2 Urinary screening tests using urine strips (n=224)

Test of	Number of negative subjects (Normal/None)	Number of positive subjects					Total positive N (%)
	-	+/-	1+	2+	3+	4+	
Protein	149 (66.52%)	51	17	7	0	0	75 (33.48%)
Glucose	216 (96.43%)	3	1	0	0	4	8 (3.57%)
Blood	224 (100%)	0	0	0	0		0 (0%)
Ketone body	224 (100%)	0	0	0	0	0	0 (0%)
Urobilinogen	224 (100%)	0	0	0	0	0	0 (0%)

Positive tests are classified as the following categories

Test of	Sensitivity	Units	Normal/None -	+/-	1+	2+	3+	4+
Protein	15 - 30	mg/dl (albumin)	-	+/-	30	100	300	1000
Glucose	75 - 125	mg/dl (glucose)	-	0.1	0.25	0.5	1	2
Blood	0.015 - 0.045	mg/dl (hemoglobin)	-	+/-	+1	2+	3+	
Ketone body	5 - 10	mg/dl (acetoacetic acids)	-	5	15	40	80	160
Urobilinogen	0.1 - 10	Ehrich unit/dl	None	0.1:normal	1	2	4	8

Table 3 Number of subjects with positive urinary protein examined by urine strips

Protein	Total	Rate(%)	Men	Rate(%)	Women	Rate(%)
Negative	149	66.52	63	60.58	86	71.67
Positive	75	33.48	41	39.42	34	28.33
Total	224	100	104	100	120	100

Quantitation of Cd

Urinary Cd concentrations in all subjects were classified into four categories as shown in Figure 5; 1) below 5 (n=52), 2) from 5 to 10 (n=79), 3) from 10 to 20 (n=56), and 4) above 20 (n=28) µg/g Cr, with a mean ± S.D. of Cd concentration of 3.95 ± 0.96, 7.14 ± 1.37, 13.63 ± 2.97 and 26.56 ± 5.12 µg/g Cr, respectively. The subjects comprised 4 sub-groups according to age; below 50 (n=68), from 50 to 60 (n=70), from 61 to 70 (n=52), and over 70 (n=30) years old. The mean ± S.D. of Cd concentration in each age group was 9.56 ± 0.87, 10.22 ± 0.92, 11.6 ± 1.22, and 9.27 ± 1.17 µg/gCr, respectively. The mean of all the urinary Cd concentrations exceeded the WHO maximum tolerable internal dose for the non-exposed population (2 µg/g Cr). There were no significant difference of Cd concentrations in these 4 groups (Figure 6). The mean ± S.D. of Cd concentration for each gender was 9.36 ± 7.06 in men and 10.97 ± 7.67 µg/g Cr in women. There was no significant difference in Cd concentrations for either sex (Figure 7). The urinary Cd concentration in all subjects is shown in Appendix E.

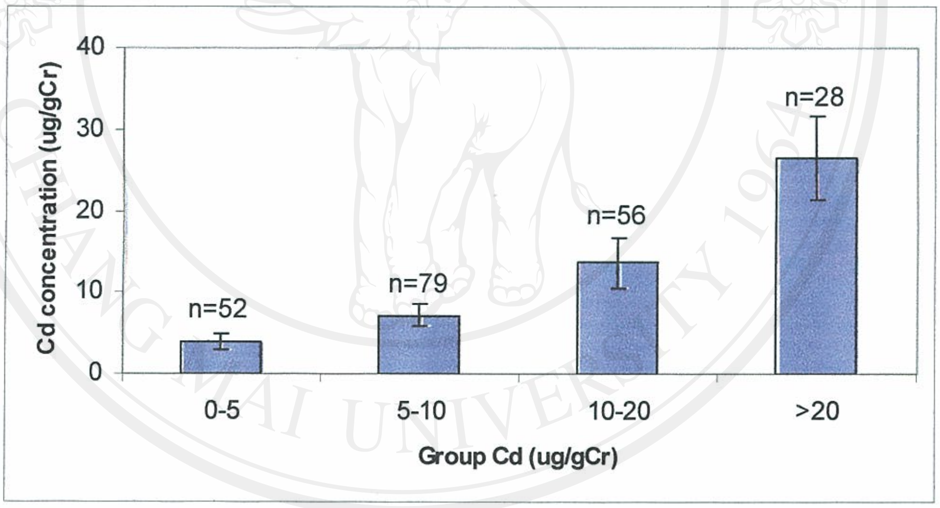


Figure 5 Four groups of urinary cadmium concentrations in the study subjects.

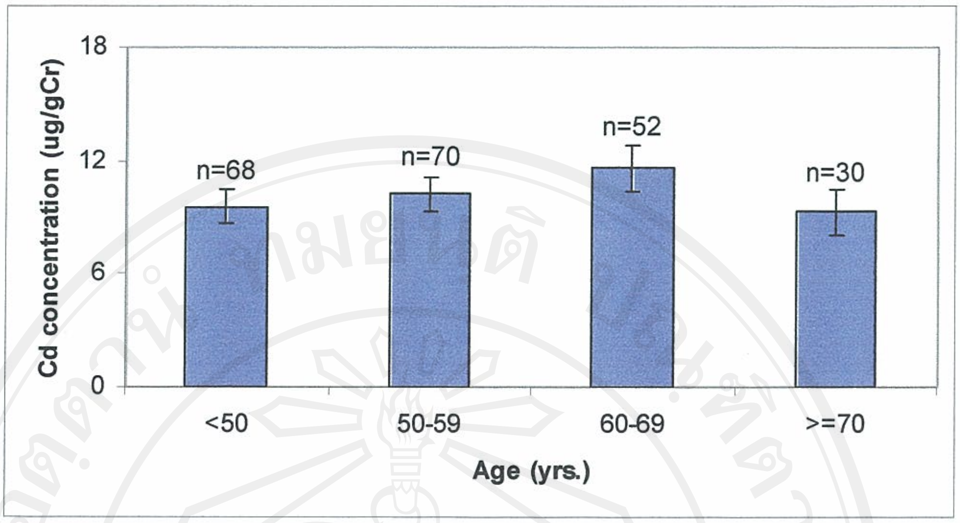


Figure 6 Urinary cadmium concentrations classified by age.

Figure 7 Urinary cadmium concentrations classified by gender.

Cd concentrations vs occupation

Farmers and non-farmers were of interest among all occupations of the study subjects. Figure 8 indicates that the urinary Cd of non-farmers was lower than that in farmers of either sex. The Cd concentration in men and women farmers was 7.8 $\pm$ 2.1 and 9.1 $\pm$ 2.1 μg/g Cr, respectively, while that in men and women non-farmers was 3.4 $\pm$ 1.8 and 5.0 $\pm$ 1.7 μg/g Cr, repectively. There was a significant difference in both genders at $p<0.05$. The urinary Cd concentration in women was higher than that in men in both groups of occupation (farmer and non-farmer). However, there was no significant difference between urinary Cd concentration in both sexes or the mean age of the subjects and their occupation as farmers.

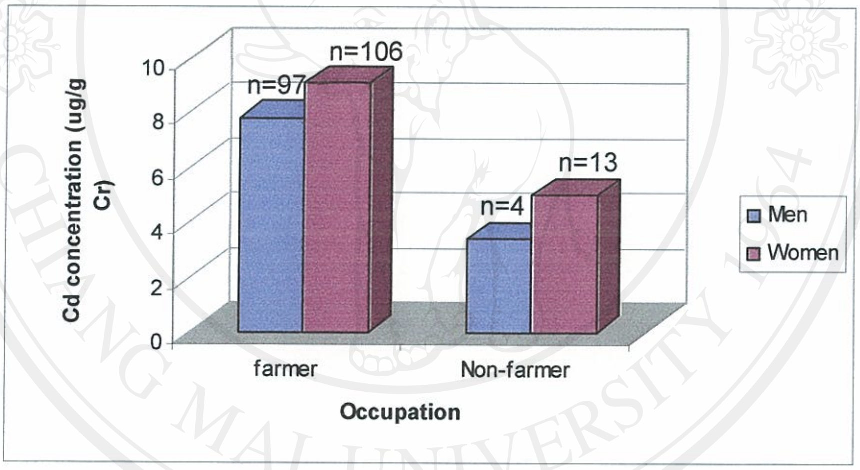


Figure 8 Cadmium concentrations classified by gender and occupations.

Cd concentrations vs smoking

Figure 9 shows urinary Cd concentrations among smoking and non-smoking subjects. It was very interesting that non-smoking women had higher Cd concentrations than smoking men. The mean ± S.D. of Cd concentration in men and women smokers was 7.6 ± 2.0 and 7.3 ± 2.0 μg/g Cr, respectively, while that in men and women non-smokers was 7.6 ± 2.2 and 9.3 ± 2.0 μg/g Cr, respectively. However, the mean Cd concentration did not differ significantly in the smokers and non-smokers of either sex. There was also no significant difference in the mean age of men subjects when comparing age with smoking habit, but there was a significant difference ($p<0.05$) in the mean age of women subjects when making the same comparison.

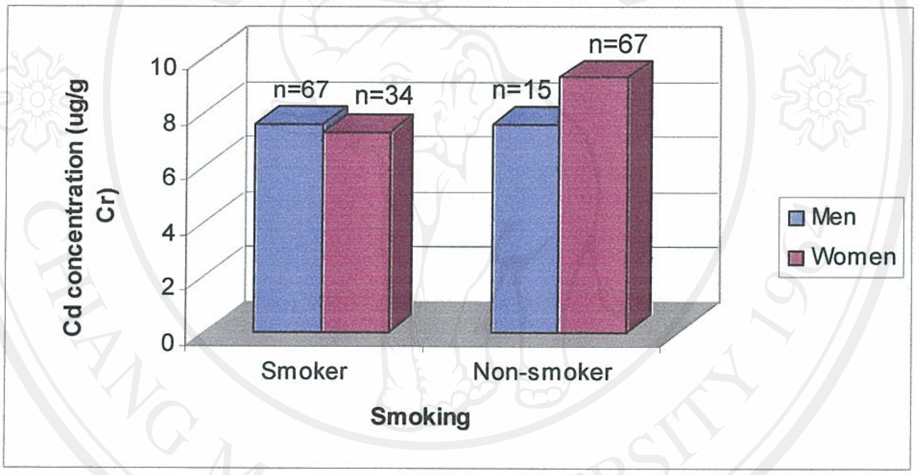


Figure 9 Cadmium concentrations classified by gender and smoking status.

Cd concentrations vs diseases

Figure 10 shows the relationship between Cd concentration and hypertension, diabetes mellitus and urinary stone.

The mean $\pm$ S.D. of Cd concentration in the subjects with hypertension was 7.6 $\pm$ 2.1 and 6.8 $\pm$ 2.0 μg/g Cr, for men and women, respectively, while that in non-hypertensive men and women was 7.5 $\pm$ 2.1 and 9.2 $\pm$ 2.0 μg/g Cr, respectively. The mean difference in Cd concentration was not statistically significant in hypertensive and non-hypertensive men. However, a high urinary Cd level in non-hypertensive women was observed. In addition, there was no significant difference in the mean age of the subjects when comparing age and hypertension.

The mean $\pm$ S.D. of Cd concentration in subjects with diabetes mellitus (DM) was 12.6 $\pm$ 2.5 and 8.4 $\pm$ 2.1 μg/g Cr in men and women, respectively, while that in men and women with no DM was 7.3 $\pm$ 2.0 and 8.6 $\pm$ 2.1 μg/g Cr, respectively. The mean difference in Cd concentration was not statistically significant in female DM and non-DM subjects, but a low urinary Cd level in male DM subjects was observed. There was a significant difference in male DM and non-DM subjects ($p<0.1$). When comparing mean age with diabetes mellitus, there was no significant difference in either sex.

Comparison of urinary Cd concentrations between subjects with and without urinary tract stone showed that the Cd concentration in men and women with urinary tract stone was 9.7 $\pm$ 2.3 and 5.7 $\pm$ 1.4 μg/g Cr, respectively, and that in men and women with no evidence of urinary tract stone was 7.3 $\pm$ 2.0 and 8.8 $\pm$ 2.1 μg/g Cr, respectively. The mean difference in Cd concentration was not statistically significant in either group of subjects. There was also no significant difference in the mean age of subjects for either sex when comparing age with evidence of urinary tract stone.

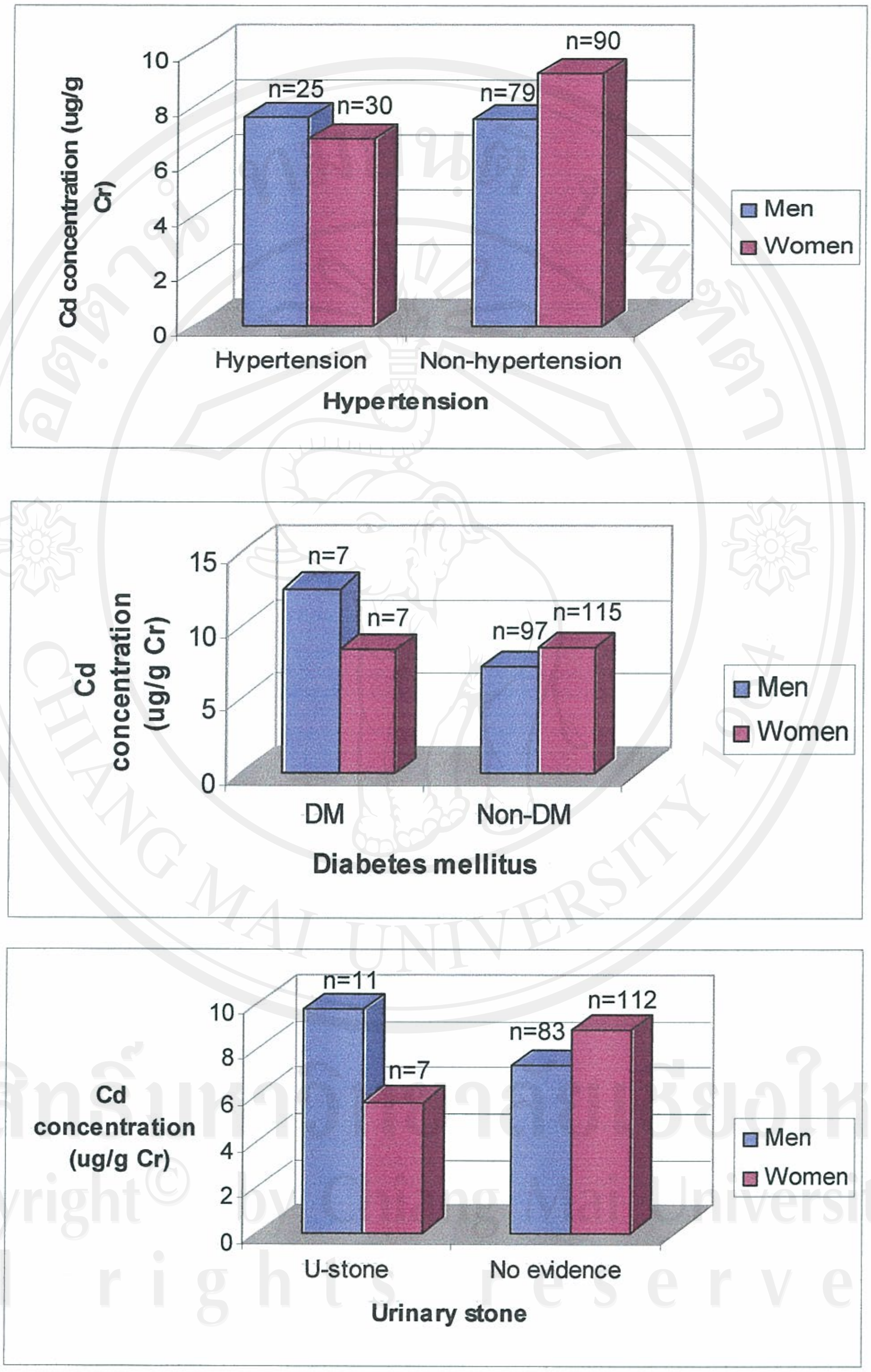


Figure 10 Cadmium concentrations classified by gender and disease status.

Quantitation of urinary calcium

Urinary Ca concentrations in the subjects were highly variable as shown in Table 4. The mean $\pm$ S.E. of Ca concentrations in all the subjects was 124.25 $\pm$ 10.63 mg/g Cr. The highest level of Ca concentration was in the aged group > 70 years old. There was no significant difference between the Ca concentrations of the 4 age groups, P=0.632 (Figure 11). The cut-off value of urinary Ca was 200 mg/g Cr. The Ca concentrations of all the subjects are shown in Appendix E.

Quantitation of urinary phosphorus

Similar to Ca concentrations, P concentrations were highly variable, with the S.D. larger than the mean, as showed in Table 5. The mean $\pm$ S.E. of P concentrations in all the subjects was 493.09 $\pm$ 47.40 mg/g Cr. The subjects were divided into 4 age groups as shown in Table 5. There was a significant difference between the age groups, < 5 and 50-59 years old, and 60-69 and $\geq$70, p<0.05 (Figure 12). The highest concentration of P was in the age group, 50-59 years. The cut-off value of urinary P was 800 mg/g Cr. The P concentrations of all subjects are shown in Appendix E.

Quantitation of urinary copper

Similar to Ca and P, urinary Cu was highly variable. The S.D. of the Cu concentrations was larger than its mean. Therefore, the mean $\pm$ S.E. of urinary Cu concentrations of all the subjects was 122.30 $\pm$ 13.75 µg/g Cr. Table 6 shows the mean, S.D. and S.E. of Cu concentrations in the four age groups. The highest level of Cu concentration was in the age group, 50-57 years old. There was no significant difference between the 4 age groups, P=0.559 (Figure 13). The cut-off value of urinary Cu was 250 µg/g Cr. The Cu concentrations of all the subjects are shown in Appendix E.

Quantitation of urinary zinc

Similar to Ca, P and Cu, Urinary Zn was highly variable. The S.D. of the Zn concentrations was larger than its mean. Therefore, the mean $\pm$ S.E. of urinary Zn concentrations of all the subjects was 616.05 $\pm$ 30.52 µg/g Cr. Table 7 shows the mean, S.D. and S.E. of Zn concentrations in the four age groups. The highest level of Zn concentration was in the age group, below 50 years old. There was no significant difference between the 4 age groups, P=0.559 (Figure 14). The cut-off value of urinary Zn was 750 µg/g Cr. The Zn concentrations of all the subjects are shown in Appendix E.

Table 4 Mean S.D. and S.E. of Ca concentrations classified by age

Age (yr)	< 50	50-59	60-69	≥70
n	68	70	52	30
Mean of Ca (mg/g Cr)	106.11	121.07	137.42	143.50
S.D.	87.55	158.37	140.47	248.85
S.E.	11.12	18.54	20.07	40.91

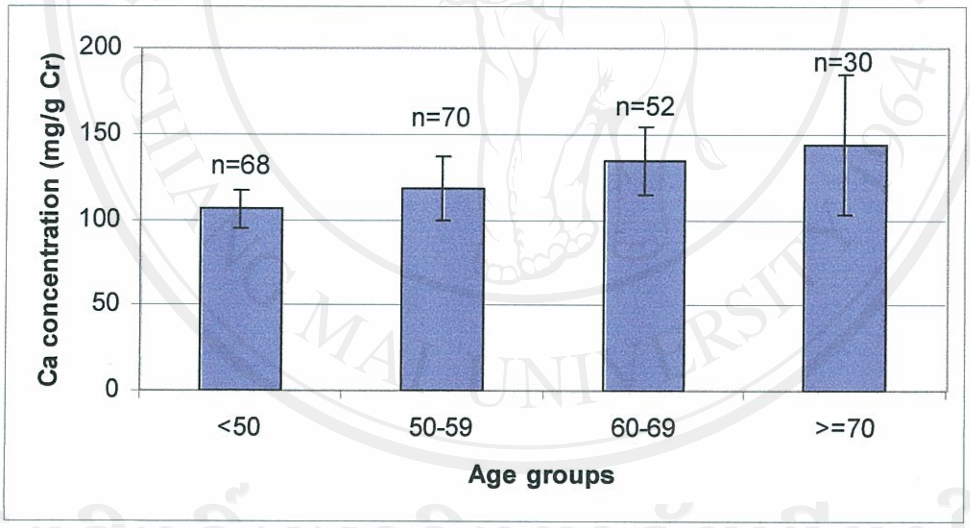
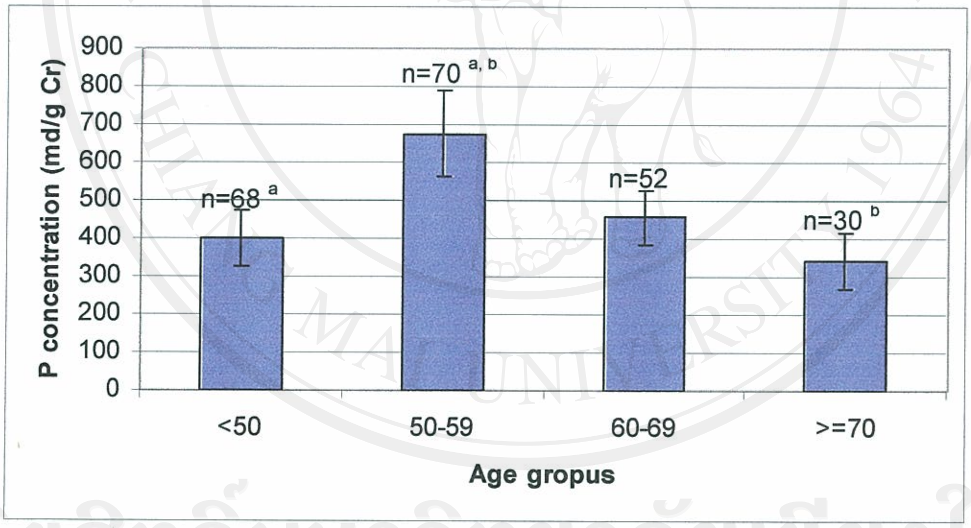


Figure 11 Urinary calcium concentrations classified by age.

Table 5 Mean S.D. and S.E. of P concentrations classified by age

Age groups	< 50	50-59	60-69	≥70
n	68	70	52	30
Mean of P (mg/g Cr)	398.89	675.55	455.70	340.46
S.D.	575.41	957.76	497.12	447.55
S.E.	73.08	112.10	71.02	73.58



a, b: $P < 0.05$

Figure 12 Urinary phosphorus concentrations classified by age.

Table 6 Mean S.D. and S.E. of Cu concentrations classified by age

Age groups	< 50	50-59	60-69	≥70
n	68	70	52	30
Mean of Cu (μg/g Cr)	128.13	144.51	96.24	102.05
S.D.	164.13	273.61	174.27	131.83
S.E.	20.84	31.59	24.90	21.67

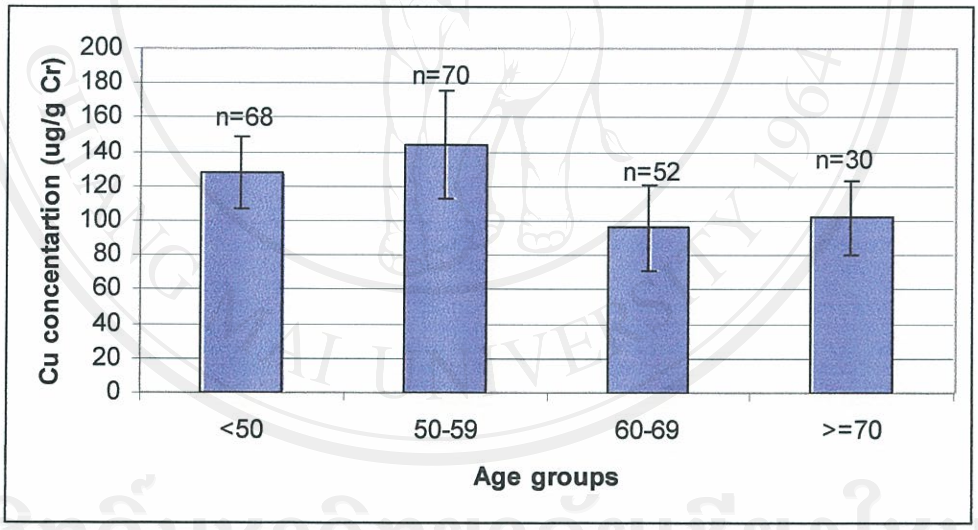


Figure 13 Urinary copper concentrations classified by age.

Table 7 Mean S.D. and S.E. of Zn concentrations classified by age

Age groups	< 50	50-59	60-69	>=70
Zn concentrations (μg/g Cr)	680.34	595.72	572.65	605.35
S.D.	669.05	427.41	234.83	209.14
S.E.	84.97	50.37	33.55	34.38

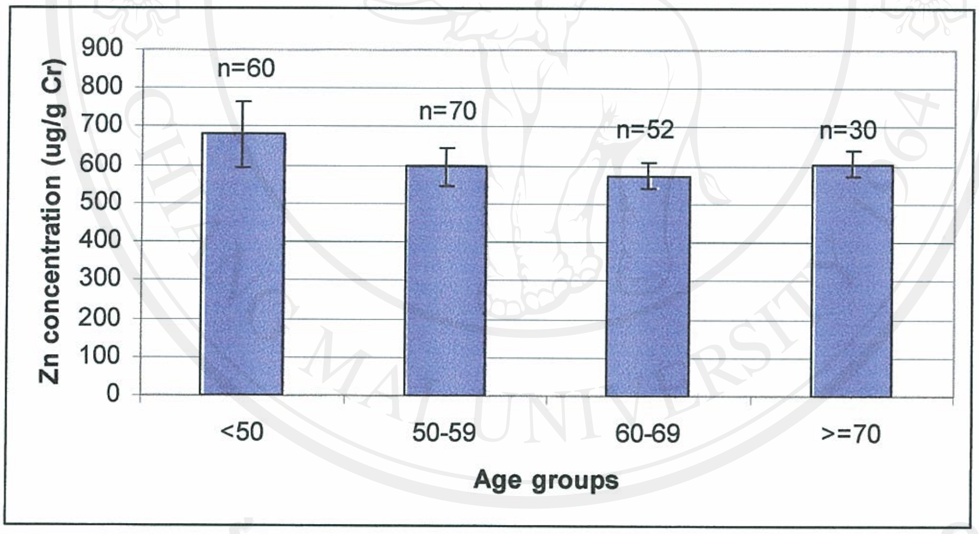


Figure 14 Urinary zinc concentrations classified by age.

Quantitation of NAG

The cut-off value of urinary NAG was 10 U/g Cr, and the range 0.7 - 35.9 U/g Cr, while the average NAG $\pm$ S.D. was 6.12 $\pm$ 4.66 U/g Cr. The NAG levels in urine were divided by concentration into 4 groups; 0-5.0, 5.1-10.0, 10.1-20 and over 20 U/g Cr, corresponding to low, average, high and very high, respectively. The relationship between urinary NAG, age and distribution in both sexes is shown in Table 8. The proportion rates of male subjects with low, average, high and very high NAG levels were 52.5%, 36.6%, 7.9% and 3%, respectively. The proportion rates of female subjects with low, average, high and very high NAG levels were 53.5%, 34.2%, 10.5% and 1.8%, respectively. The total percentage of NAG in the subjects with high levels was 10.9% in men and 12.3% in women. The mean age of the subjects with high urinary excretion of NAG was over 60 years old, but there were also younger subjects below 40's years old experiencing the same.

Quantitation of β_2-MG

The range of urinary β_2-MG was 10.9 - 118,882 μg/g Cr. The average β_2-MG $\pm$ S.D. was 5,231 $\pm$ 17,285 μg/g Cr. The urinary β_2-MG was divided into 4 groups according to the cut-off level of β_2-MG (1,000 μg/g Cr) in Japanese people, who lived in a Cd polluted area. β_2-MG in urine at concentrations of 0-300, 301-1,000, 1,001-10,000 and over 10,000 μg/g Cr corresponded to low, average, high and very high levels, respectively. The relationship of urinary β_2-MG, age and distribution in both sexes is shown in Table 9. The proportion rates of male subjects with low, average, high and very high β_2-MG were 54.5%, 13.9%, 20.8% and 10.9%, respectively. The proportion rates of female subjects with low, average, high and very high β_2-MG were 64.0%, 16.7%, 13.2% and 6.1%, respectively. The total percentage of β_2-MG in all subjects with high levels was 31.7% in men and 19.3% in women. The relationship between β_2-MG and age was similar to that found between NAG and age.

Quantitation of α_1-MG

The range of urinary α_1-MG was 0.49 - 100.50 μg/g Cr. The average α_1-MG $\pm$ S.D. was 11.35 $\pm$ 14.96 μg/g Cr. The urinary α_1-MG was divided into 4 groups according to the cut-off level of α_1-MG (15 μg/g Cr) in Japanese people, who lived in a Cd polluted area. The α_1-MG in urine at concentrations of 0-15, 15.1-30, 30.1-45 and over 45 μg/g Cr corresponded to low, average, high and very high levels, respectively. The relationship of urinary α_1-MG, age and distribution in both sexes is shown in Table 10. The proportion rates of male subjects with low, average, high and very high α_1-MG were 71.3%, 17.8%, 4.0% and 6.9%, respectively. The proportion rates of female subjects with low, average, high and very high α_1-MG were 84.22%, 12.28%, 1.75% and 1.75%, respectively. The total percentage of α_1-MG in all subjects with high levels was 28.7% in men and 15.8% in women. The relationship between α_1-MG and age was similar to that found between NAG and age.

Quantitation of albumin

The range of urinary albumin was 0.2 - 518.2 mg/g Cr. The average albumin $\pm$ S.D. was 23.24 $\pm$ 55.06 mg/g Cr. The urinary albumin was divided into 4 groups according to the cut-off level of albumin (30 mg/g Cr) in Japanese people, who lived in a Cd polluted area. Albumin in urine at concentrations of 0-15, 15.1-30, 30.1-45 and over 45 mg/g Cr corresponded to low, average, high and very high levels, respectively. The relationship of urinary albumin, age and distribution in both sexes is shown in Table 11. The proportion rates of male subjects with low, average, high and very high albumin were 87.1%, 5.9%, 1.0% and 5.9%, respectively. The proportion rates of female subjects with low, average, high and very high albumin were 83.3%, 7.9%, 5.3% and 3.5%, respectively. The total percentage of albumin in all subjects with high levels was 12.9% in men and 16.7% in women. The relationship between albumin and age was similar to that found between NAG and age.

Quantitation of total protein

The range of urinary total protein was 0 - 1,211.38 mg/g Cr. The average total protein $\pm$ S.D. was 90.99 $\pm$ 185.08 mg/g Cr. The urinary total protein was divided into 4 groups according to its upper limit of measurement of 100 mg/g Cr or more. Total protein in urine at concentrations of 0-50, 50.1-100, 100.1-150 and over 150 mg/g Cr corresponded to low, average, high and very high levels, respectively. The relationship of urinary total protein, age and distribution in both sexes is shown in Table 12. The proportion rates of male subjects with low, average, high and very high total protein were 69.3%, 12.9%, 2.0% and 15.8%, respectively. The proportion rates of female subjects with low, average, high and very high total protein were 73.7%, 11.4%, 2.6% and 12.3%, respectively. The total percentage of total protein in all subjects with high levels was 17.8% in men and 14.9% in women. The relationship between total protein and age was similar to that found between NAG and age.

Quantitation of total amino nitrogen

The range of urinary total amino nitrogen was 46.6 – 431.7 μg/g Cr. The average total amino nitrogen $\pm$ S.D. was 124.87 $\pm$ 51.20 μg/g Cr. The urinary total amino nitrogen was divided into 4 groups according to its upper limit of measurement of 180 μg/g Cr or more. Total amino nitrogen in urine at concentrations of 0-90, 90.1-180, 180.1-270 and over 270 μg/g Cr corresponded to low, average, high and very high levels, respectively. The relationship of urinary total amino nitrogen, age and distribution in both sexes is shown in Table 13. The proportion rates of male subjects with low, average and high total amino nitrogen were 20.79%, 76.24% and 2.97%, respectively. The proportion rates of female subjects with low, average, high and very high total amino nitrogen were 17.70%, 69.03%, 7.96% and 5.31%, respectively. The total percentage of total amino nitrogen in all subjects with high levels was 2.97% in men and 13.27% in women. The relationship between total amino nitrogen and age was similar to that found between NAG and age.

Quantitation of lysozyme

The range of urinary lysozyme was 0.03 - 1,108.77 mg/g Cr. The average lysozyme ± S.D. was 30.74 ± 126.96 mg/g Cr. The urinary lysozyme was divided into 4 groups according to its upper limit of measurement of 4 mg/g Cr or more. Lysozyme in urine at concentrations of 0-2, 2.1-4, 4.1-6 and over 6 mg/g Cr corresponded to low, average, high and very high levels, respectively. The relationship of urinary lysozyme, age and distribution in both sexes is shown in Table 14. The proportion rates of male subjects with low, average, high and very high lysozyme were 83.3%, 10.0%, 3.3% and 3.3%, respectively. The proportion rates of female subjects with low, average, high and very high lysozyme were 83.1%, 1.7%, 1.7% and 13.6%, respectively. The total percentage of lysozyme in all subjects with high levels was 6.7% in men and 15.3% in women. Most of the subjects had lysozyme under the detection limit (126 people; 66 men and 60 women). The relationship between lysozyme and age was similar to that found between NAG and age.

Quantitation of glucose

The range of urinary glucose was 0 - 18,653.03 mg/g Cr. The average glucose ± S.D. was 381.85 ± 2,165.75 mg/g Cr. The urinary glucose was divided into 4 groups according to its upper limit of measurement of 200 mg/g Cr or more. Glucose in urine at concentrations of 0-100, 100.1-200, 200.1-300 and over 300 mg/g Cr corresponded to low, average, high and very high levels, respectively. The relationship of urinary glucose, age and distribution in both sexes is shown in Table 15. The proportion rates of male subjects with low, average and very high glucose were 93.1%, 1.0% and 5.9%, respectively. The proportion rates of female subjects with low, average, high and very high glucose were 85.1%, 5.3%, 0.9% and 8.8%, respectively. The total percentage of glucose in all subjects with high levels was 5.9% in men and 9.7% in women. The relationship between glucose and age was similar to that found between NAG and age.

Table 8 Distribution of urinary NAG classified by gender

Levels	Total N (%)	NAG in men			NAG in women		
		N (%)	Mean ± S.D.		N (%)	Mean ± S.D.	
			Urinary NAG	Age		Urinary NAG	Age
Low	64 (29.8%)	53 (52.5%)	3.2 ± 1.1	58.5 ± 40.3	61 (53.5%)	3.5 ± 1.0	62.0 ± 31.1
Average	76 (35.3%)	37 (36.6%)	6.9 ± 1.3	60.5 ± 27.6	39 (34.2%)	7.2 ± 1.5	64.5 ± 27.6
High	20 (9.3%)	8 (7.9%)	12.6 ± 1.7	53.5 ± 27.6	12 (10.5%)	13.2 ± 2.5	63.5 ± 20.5
Very high	5 (2.3%)	3 (3%)	27.3 ± 7.5	67.5 ± 6.4	2 (1.8%)	29.2 ± 0.1	58.0 ± 16.9
Total	215 (100%)	101 (100%)	6.1 ± 4.8	58.5 ± 40.3	114 (100%)	6.2 ± 4.5	62.0 ± 31.1

NAG: N-acetyl-β-D-glucosaminidase
U/g Cr: Unit per gram creatinine
Mean ± S.D.: Average ± standard deviation
N: Number of subjects

Table 9 Distribution of urinary β_2-MG classified by gender

Levels	Total N (%)	β_2-MG in men			β_2-MG in women		
		N (%)	Mean $\pm$ S.D.		N (%)	Mean $\pm$ S.D.	
			Urinary β_2-MG	Age		Urinary β_2-MG	Age
Low	128 (59.5%)	55 (54.5%)	90 $\pm$ 67	53 $\pm$ 33	73 (64.0%)	104 $\pm$ 68	60.0 $\pm$ 28.3
Average	33 (15.3%)	14 (13.9%)	862 $\pm$ 1,466	61 $\pm$ 27	19 (16.7%)	550 $\pm$ 190	61.0 $\pm$ 24.0
High	36 (16.7%)	21 (20.8%)	3,715 $\pm$ 2,281	66 $\pm$ 29	15 (13.2%)	3,524 $\pm$ 2,585	63.5 $\pm$ 19.1
Very high	18 (8.4%)	11 (10.9%)	45,481 $\pm$ 35,481	63 $\pm$ 18	7 (6.1%)	45,328 $\pm$ 37,248	65.0 $\pm$ 26.9
Total	215 (100%)	101 (100%)	6,178 $\pm$ 18,470	61 $\pm$ 27	114 (100%)	4,403 $\pm$ 16,212	62.0 $\pm$ 31.1

β_2-MG: β_2-microglobulin
μg/g Cr: microgram per gram creatinine
Mean $\pm$ S.D.: Average $\pm$ standard deviation
N: Number of subjects

Table 10 Distribution of urinary α_1-MG classified by gender

Levels	Total N (%)	α_1-MG in men			α_1-MG in women		
		N (%)	Mean $\pm$ S.D.		N (%)	Mean $\pm$ S.D.	
			Urinary α_1-MG	Age		Urinary α_1-MG	Age
Low	168 (78.1%)	72 (71.3%)	6.33 $\pm$ 3.83	53.75 $\pm$ 12.13	96 (84.22%)	5.45 $\pm$ 3.57	54.91 $\pm$ 8.81
Average	32 (14.9%)	18 (17.8%)	19.13 $\pm$ 3.03	61.50 $\pm$ 11.63	14 (12.28%)	20.69 $\pm$ 3.56	60.62 $\pm$ 10.10
High	6 (2.8%)	4 (4.0%)	36.66 $\pm$ 5.04	66.50 $\pm$ 11.62	2 (1.75%)	43.95 $\pm$ 1.28	67.00 $\pm$ 24.04
Very high	9 (4.2%)	7 (6.9%)	59.76 $\pm$ 18.46	69.43 $\pm$ 7.93	2 (1.75%)	97.50 $\pm$ 4.23	72.00 $\pm$ 9.90
Total	215 (100%)	101 (100%)	13.44 $\pm$ 15.67	58.05 $\pm$ 12.33	114 (100%)	9.48 $\pm$ 14.10	56.07 $\pm$ 9.64

α_1-MG: α_1-microglobulin
μg/g Cr: microgram per gram creatinine
Mean $\pm$ S.D.: Average $\pm$ standard deviation
N: Number of subjects

Table 11 Distribution of urinary albumin classified by gender

Levels	Total N (%)	Alb in men			Alb in women		
		N (%)	Mean ± S.D.		N (%)	Mean ± S.D.	
			Urinary Alb	Age		Urinary Alb	Age
Low	183 (85.1%)	88 (87.1%)	7.34 ± 6.46	57.4 ± 12.11	95 (83.3%)	8.29 ± 6.24	54.52 ± 8.63
Average	15 (7.0%)	6 (5.9%)	44.33 ± 9.69	58.84 ± 14.60	9 (7.9%)	36.67 ± 7.84	69.13 ± 8.58
High	7 (3.3%)	1 (1.0%)	78.25 ± 0.35	58.50 ± 6.36	6 (5.3%)	79.02 ± 5.99	61.33 ± 11.66
Very high	10 (4.7%)	6 (5.9%)	175.30 ± 60.70	69.33 ± 13.91	4 (3.5%)	321.15 ± 136.20	60.25 ± 12.18
Total	215 (100%)	101 (100%)	21.15 ± 43.60	58.05 ± 12.33	114 (100%)	25.13 ± 63.80	56.12 ± 9.67

Alb: albumin
mg/g Cr: microgram per gram creatinine
Mean ± S.D.: Average ± standard deviation
N: Number of subjects

Table 12 Distribution of urinary total protein classified by gender

Levels	Total N (%)	Total protein in men			Total protein in women		
		N (%)	Mean ± S.D.		N (%)	Mean ± S.D.	
			Urinary TP	Age		Urinary TP	Age
Low	154 (71.6%)	70 (69.3%)	20.10 ± 12.15	57.4 ± 12.11	84 (73.7%)	20.69 ± 11.76	54.52 ± 8.63
Average	26 (12.1%)	13 (12.9%)	98.82 ± 120.75	58.84 ± 14.60	13 (11.4%)	66.87 ± 16.80	69.13 ± 8.58
High	5 (2.3%)	2 (2.0 %)	114.36 ± 14.14	58.50 ± 6.36	3 (2.6%)	118.29 ± 20.03	61.33 ± 11.66
Very high	30 (14.0%)	16 (15.8%)	431.78 ± 317.49	69.33 ± 13.91	14 (12.3%)	381.11 ± 326.01	60.25 ± 12.18
Total	215 (100%)	101 (100%)	98.65 ± 195.32	58.05 ± 12.33	114 (100%)	84.55 ± 176.16	56.12 ± 9.67

TP: total protein
mg/g Cr: milligram per gram creatinine
Mean ± S.D.: Average ± standard deviation
N: Number of subjects

Table 13 Distribution of urinary total amino nitrogen classified by gender

Levels	Total N (%)	Total amino nitrogen in men			Total amino nitrogen in women		
		N (%)	Mean ± S.D.		N (%)	Mean ± S.D.	
			Urinary AminoN	Age		Urinary AminoN	Age
Low	41 (19.1%)	21 (20.79%)	76.57 ± 12.88	62.24 ± 10.97	20 (17.70%)	77.24 ± 10.83	56.25 ± 11.24
Average	155 (72.1%)	77 (76.24%)	116.80 ± 21.70	57.75 ± 12.63	78 (69.03%)	128.77 ± 23.01	56.34 ± 9.40
High	12 (5.6%)	3 (2.97%)	214.53 ± 39.16	44.67 ± 10.07	9 (7.96%)	203.06 ± 25.17	56.44 ± 10.42
Very high	30 (14.0%)	-	-	-	6 (5.31%)	340.75 ± 71.44	59.67 ± 10.86
Total	215 (100%)	101 (100%)	111.52 ± 31.76	58.28 ± 12.51	113 (100%)	136.42 ± 61.21	56.53 ± 9.77

AminoN: total anino nitrogen
μg/g Cr: microgram per gram creatinine
Mean ± S.D.: Average ± standard deviation
N: Number of subjects

Table 14 Distribution of urinary lysozyme classified by gender

Levels	Total N (%)	LYZ in men			LYZ in women		
		N (%)	Mean ± S.D.		N (%)	Mean ± S.D.	
			Urinary LYZ	Age		Urinary LYZ	Age
Low	74 (83.1%)	25 (83.3%)	0.43 ± 0.53	59.68 ± 10.78	49 (83.1%)	0.59 ± 0.48	55.84 ± 9.5
Average	4 (4.5%)	3 (10.0%)	3.06 ± 0.15	63.00 ± 2.83	1 (1.7%)	2.02 ± 0	66.00 ± 0
High	2 (2.2%)	1 (3.3%)	4.21 ± 0.98	78.00 ± 2.83	1 (1.7%)	4.95 ± 0	72.00 ± 0
Very high	9 (10.1%)	1 (3.3%)	193.73 ± 375.21	69.88 ± 9.48	8 (13.6)	167.54 ± 126.24	61.88 ± 11.18
Total	89 (100%)	30 (100%)	42.57 ± 183.99	63.05 ± 11.23	59 (100%)	23.32 ± 72.41	57.10 ± 9.77

LTZ: lysozyme
mg/g Cr: milligram per gram creatinine
Mean ± S.D.: Average ± standard deviation
N: Number of subjects

Table 15 Distribution of urinary glucose classified by gender

Levels	Total N (%)	Glu in men			Glu in women		
		N (%)	Mean ± S.D.		N (%)	Mean ± S.D.	
			Urinary Glu	Age		Urinary Glu	Age
Low	191 (88.8%)	94 (93.1%)	18.46 ± 19.84	58.15 ± 12.38	97 (85.1%)	18.84 ± 20.03	56.42 ± 9.90
Average	156 (72.6%)	1 (1.0%)	126.92 ± 0	64.0 ± 0	6 (5.3%)	135.91 ± 24.82	58.00 ± 11.27
High	1 (0.5%)	-	-	-	1 (0.9%)	227.65 ± 0	63.00 ± 0
Very high	16 (7.4%)	6 (5.9%)	6,710.21 ± 9,209.85	59.67 ± 16.45	10 (8.8%)	3,844.49 ± 4,853.93	52.3 ± 5.88
Total	215 (100%)	101 (100%)	409.33 ± 2,576.47	58.29 ± 12.51	114 (100%)	357.50 ± 1,732.75	56.26 ± 9.68

Glu: glucose
mg/g Cr: milligram per gram creatinine
Mean ± S.D.: Average ± standard deviation
N: Number of subjects

Relationship between Cd concentrations and other metals (Ca, P, Cu and Zn) and urinary renal markers

Cd and other metals (Ca, P, Cu and Zn)

Concentrations of urinary Zn, Cu, Ca and P, classified by Cd concentration, are shown in Table 16. No statistically significant differences were found. The mean $\pm$ S.E. of urinary Zn tended to increase in proportion to Cd concentration except in the group of Cd > 20 µg/g Cr. The mean $\pm$ S.E. of urinary Cu was non-proportional to the elevation of Cd concentration. The mean $\pm$ S.E. of urinary Ca was no different to all groups of Cd concentration. The mean $\pm$ S.E. of urinary P was non-proportional to the elevation of Cd concentration.

Cd and renal markers (NAG, β_2-MG, α_1-MG, Alb, TP, AminoN, LYZ and Glu)

The correlation analysis that was calculated to investigate the relationship between urinary Cd and urinary markers of renal dysfunction is shown in Table 17. All correlations were positive and statistically significant, except for α_1-MG and albumin in women, and total amino nitrogen and glucose in both sexes. Urinary lysozyme could not be statistically significant because most of the data were below its detection limit (data not shown). The correlation between urinary Cd and NAG was higher than other renal markers.

The mean $\pm$ S.D. of urinary markers for renal dysfunction among 4 urinary Cd categories; below 5 (n = 52), from 5 to 10 (n = 79), from 10 to 20 (n = 56), and above 20 (n = 28) µg/g Cr is shown in Table 18. The mean β_2-MG in the urinary Cd category above 20 and from 5-10 µg/gCr were significantly higher than that in urinary Cd category below 5 µg/g Cr ($P<0.001$, $P<0.05$). Likewise, the mean NAG was significantly higher in the urinary Cd categories above 20, from 10 to 20, and from 5 to 10 than in the urinary Cd category below 5 µg/g Cr ($P<0.001$, $P<0.01$). The mean α_1-MG was significantly higher in the urinary Cd category above 20 than that in the urinary Cd category below 5 µg/g Cr ($P<0.05$). There were no significant differences among urinary Cd categories of the other renal markers. These results indicated that there were three urinary protein markers; β_2-MG, NAG and α_1-MG, that were significantly different according to urinary Cd concentrations. Comparison of the positive rates of renal markers between 4 urinary Cd categories is shown in Figure 15.

The results showed that the positive rate of β_2-MG, NAG, and α_1-MG increased if urinary Cd concentrations increased. These findings indicated that β_2-MG, NAG, and α_1-MG were more sensitive than other renal markers. β_2-MG and α_1-MG also had a wide range and increased in proportion to urinary Cd concentration. NAG was a very good marker for showing the difference in Cd concentrations between subjects who lived in the polluted (urinary Cd $\geq$5 µg/g Cr) and non-polluted (urinary Cd 0-5 µg/g Cr) areas.

Total protein concentration was non-proportional to the elevation of Cd concentration. It was similar to α_1-MG. Total amino nitrogen concentration was different in only high Cd concentrations (>20 µg/g Cr). Lysozyme was slightly different in the urine between subjects with a Cd concentration of below and above 5 µg/g Cr. Urinary glucose showed no difference in all groups. The differences of Total protein positive rates among these subjects in all Cd categories were non-proportional to urinary Cd concentrations. There was a similar relationship of total protein positive rates to urinary Cd as that of α_1-MG positive rates. Total amino nitrogen positive rates in the subjects with a high Cd concentration (>20 μg/g Cr) were higher than those in subjects with other Cd concentrations. The lysozyme positive rate was slightly different between subjects with a Cd concentration below and above 5 μg/g Cr. Glucose concentrations showed no difference in relationship with Cd concentrations in all subjects in any of the Cd concentration categories.

Metals (Cd, Zn, Cu, Ca and P) and renal markers

Table 19 showed the correlation of tubular dysfunction markers and five elements in urine such as Cd, Ca, P, Cu and Zn. The results indicated that most of the renal markers were correlated more positively with Cd concentrations than other metals. Urinary Zn showed positive correlations with urinary NAG and glucose. No correlation was found in renal markers and urinary Cu, Ca and P. Therefore, urinary protein markers; NAG, β_2-MG and α_1-MG, correlated with Cd concentrations more than with other metals.

Conclusions

All of these results showed that the subjects who lived in Mae Sot district, Tak province, Thailand had a high risk of renal dysfunction, especially those who were farmers. The average urinary Cd concentrations of all subjects exceeded the WHO maximum tolerable internal dose for a non-exposed population. Qualitative and quantitative analyses of the renal dysfunction markers showed a positive correlation to high urinary Cd concentrations, especially in NAG, β_2-MG and α_1-MG. β_2-MG and α_1-MG had a wide range and increased proportionally to urinary Cd concentrations. However, NAG was a better marker for the early detection of renal dysfunction related to Cd exposure than other renal proteins.

Table 16 Urinary excretion concentrations of Zn, Cu, Ca and P classified by Cd concentration

Cd group	Urinary excretion concentration for metals							
	Zn (μg/g Cr)		Cu (μg/g Cr)		Ca (mg/g Cr)		P (mg/g Cr)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
0-5	550.09	44.47	130.81	36.96	108.36	12.39	366.21	49.78
>5-10	648.87	57.46	188.77	32.06	103.62	10.43	358.72	31.05
>10-20	672.12	71.58	149.26	26.6	107.99	11.08	391.77	59.08
>20	574.16	47.25	171.47	39.76	104.82	11.89	496.23	118.82
All	621.45	30.82	162.88	17.36	106.06	5.84	386.6	27.15

Table 17 Correlation coefficients to urinary cadmium of renal biomarkers

Correlation coeffient	Renal biomarkers						
	NAG(U/g Cr)	β_2-MG(μg/g Cr)	α_1-MG(μg/gCr)	TP(mg/g Cr)	Alb(mg/g Cr)	AminoN(mg/l)	Glu(mg/l)
U-Cd (male)	0.460**	0.324**	0.365**	0.193	0.196*	-0.025	-0.082
U-Cd (female)	0.254**	0.291**	0.168	-0.181	-0.093	0.153	-0.15
Correlation coefficient controlling for age (age ≥ 50 years)							
U-Cd (male)	0.365**	0.352**	0.332**	0.156	0.202	0.022	-0.107
U-Cd (female)	0.227*	0.364**	0.199	-0.053	-0.121	0.162	-0.09

Correlation coefficients were tested by Spearman's rho

*$P < 0.05$, **$P < 0.01$

NAG: N-acetyl-β-D-glucosaminidase, β_2-MG: β_2-microglobulin, α_1-MG: α_1-microglobulin, TP: total protein, Alb: total protein, AminoN: total amino nitrogen, Glu: glucose

Table 18 Comparison of urinary markers for renal tubular dysfunction classified by Cd levels

Markers	Unit	Urinary Cd (mean ± S.D., μg/gCr)			
		0-5 (n = 52)	>5-10 (n = 79)	>10-20 (n = 56)	> 20 (n = 28)
β_2-MG	μg/gCr	158.5 ± 6.9	336.9 ± 8.3	397.1 ± 8.8	1433.2 ± 10.2***[1]
NAG	U/gCr	3.6 ± 1.9	5.3 ± 1.8**	5.3 ± 1.7**	6.9 ± 1.5***
α_1-MG	μg/gCr	4.9 ± 2.6	7.2 ± 2.8	7.0 ± 2.7	10.4 ± 2.6*
AminoN	μg/gCr	113.1 ± 1.4	115.1 ± 1.4	122.3 ± 1.4	126.5 ± 1.4
Alb	mg/gCr	7.9 ± 2.8	10.2 ± 4.3	7.6 ± 3.0	11.3 ± 2.9
Protein	mg/gCr	17.0 ± 5.7	21 ± 7.4	24.2 ± 5.2	15.9 ± 10.1
Glucose	mg/gCr	17.9 ± 5.9	15.5 ± 7.1	10.6 ± 11.7	13.1 ± 11.3

*: P<0.05, **: P<0.01 ***: P<0.001 as compared to the group with a urinary Cd of 0-5 μg/g Cr

[1]: P<0.05 as compared to the group with a urinary Cd of 5-10 μg/g Cr

NAG: N-acetyl-β-D-glucosaminidase, β_2-MG: β_2-microglobulin, α_1-MG: α_1-microglobulin, Alb: total protein, Prot: protein, AminoN: total amino nitrogen, Glu: glucose, Cr: creatinine

The number of subjects in each group; below 5 (n = 52), from 5 to 10 (n = 79), from 10 to 20 (n = 56), and above 20 (n = 28) μg/g Cr

Using statistic ANOVA

Table 19 Correlation of tubular dysfunction markers and four elements in urine

Tubular dysfunction marker	Cd (n=224)	Ca (n=224)	P (n=224)	Zn (n=218)	Cu (n=168)
β_2-MG (n=224)	0.310**	0.047	0.077	0.007	0.089
α_1-MG (n=224)	0.241**	0.050	0.083	0.111	0.115
NAG (n=224)	0.353**	0.077	0.067	0.189**	0.132
Alb (n=224)	0.052	-0.050	0.096	0.133	-0.025
TP (n=223)	-0.022	-0.088	0.004	0.089	0.102
AminoN (n=224)	0.123	0.014	-0.080	0.129	0.002
LYZ (n=96)	0.102	0.095	-0.034	0.106	0.163
Glu (n=224)	0.105	-0.060	0.011	0.143*	0.136

* $P < 0.05$, ** $P < 0.01$ (by using log transform and pearson correlation test)
NAG: N-acetyl-β-D-glucosaminidase, β_2-MG: β_2-microglobulin, α_1-MG: α_1-microglobulin, Alb: albumin, TP: total protein, AminoN: total amino nitrogen, LYZ: lysozyme, Glu: glucose, Cd: cadmium, Ca: calcium, P: phosphorus, Zn: zinc, Cu: copper

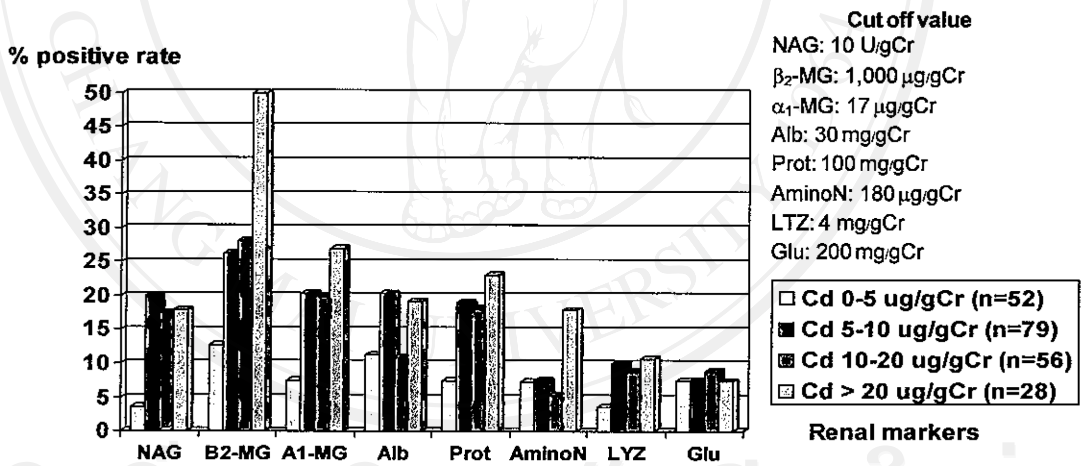


Figure 15 Positive rates of urinary markers for renal dysfunction classified by Cd concentration.

NAG: N-acetyl-β-D-glucosaminidase, β_2-MG: β_2-microglobulin, α_1-MG: α_1-microglobulin, Alb: total protein, Prot: protein, AminoN: total amino nitrogen, LTZ: lysozyme, Glu: glucose, Ca: calcium, P: phosphorus, Cu: copper, Zn: zinc, Cr: creatinine