Longitudinal Association between Selenium Levels and Hypertension in a Rural Elderly Chinese Cohort

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Abstract

Objectives: Results from previous studies have been inconsistent on the association between selenium and hypertension, and very few studies on this subject have focused on the elderly population. The purpose of this study is to examine the relationship between selenium level and hypertension in a rural elderly Chinese cohort.

Design: A longitudinal study was implemented and data were analyzed using logistic regression models and Cox proportional hazards regression model adjusting for potential confounders. The association between selenium level and prevalent hypertension at baseline and between selenium and incident hypertension were examined.

Setting: Community-based setting in four rural areas in China.

Subjects: A total of 2000 elderly aged 65 years and over (mean 71.9±5.6 years) participated in this study.

Measurements: Nail selenium levels were measured in all subjects at baseline. Blood pressure measures and self-reported hypertension history were collected at baseline, 2.5 years and 7 years later. Hypertension was defined as systolic blood pressure 140 mmHg or higher, diastolic blood pressure 90 mmHg or higher, or reported use of anti-hypertensive medication.

Results: The rate of baseline hypertension was 63.50% in this cohort and the mean nail selenium level is 0.413±0.183μg/g. Multi-covariate adjusted cross-sectional analyses indicated that higher selenium level was associated with higher blood pressure measures at baseline and higher rates of hypertension. For the 635 participants with normal blood pressure at baseline, 360 had developed hypertension during follow-up. The incidence rate for hypertension was 45.83%, 52.27, 62.50%, 70.48%, and 62.79% from the first selenium quintile to the fifth quintile respectively. Comparing to the lowest quintile group, the hazard ratio were 1.41 (95%CI: 1.03

to1.94), 1.93 (95%CI: 1.40 to 2.67), 2.35 (95%CI: 1.69 to 3.26) and 1.94 (95%CI: 1.36 to 22.77) for the second selenium quintile to the fifth quintile respectively.

Conclusions: Our findings suggest that high selenium may play a harmful role in the development of hypertension. Future studies are needed to confirm our findings and to elucidate a plausible biological mechanism.

Keywords: Selenium; Hypertension; Blood pressure; Elderly population; Environmental exposure

Introduction

Hypertension affects nearly one billion people worldwide, many of them over the age of 65, and contributes to the global burden of disease and mortality [1]. Studies have shown that oxidative stress is one of the fundamental mechanisms underlying hypertension [2]. Antioxidants inhibit oxidation reactions, thereby reducing the number of free radicals produced and the amount of damage they can cause. Selenium, an essential trace element with antioxidant properties [3], was hypothesized to have a protective effect on hypertension [4].

A link between selenium and hypertension was provided in selenium's role in Keshan disease, a disorder that occurred in regions of China where selenium was severely deficient in the soil and diet, the symptoms of Keshan disease, including hypertension, can be relieved by administering selenium supplements [5]. There are reports from observational studies of higher selenium levels associated with lower blood pressure [6-8]. However, other studies have shown that in populations where selenium levels exceed the recommended daily intake, higher selenium levels were found to be associated with higher blood pressure and higher rates of hypertension [9-11]. It should be noted that few of published studies are longitudinal in design. In addition to the inconsistency in findings on the relationship between selenium and blood pressure, very few studies on this subject have focused on the elderly population despite the considerable proportion of the elderly suffering from hypertension. The objective of this study was to examine the relationship between selenium level and hypertension in an elderly Chinese population.

Materials and methods

Study design and participants

Participants were recruited for the Selenium and Cognitive Decline study, a longitudinal epidemiologic project funded by the National Institute of Health examining the long-term impact

of selenium on cognitive decline in rural elderly Chinese. Participants were enrolled between 2003 and 2005, and underwent two follow-up evaluations at 2.5 years and 7 years after baseline. Two counties from Sichuan Province in southwestern China, and two counties from Shandong Province in eastern China were selected for the project. These study sites were selected for the following reasons: (1). They were rural counties; (2). The two counties within a province differed in selenium levels but were similar in demographics and other trace element measures; and (3). The four counties were also required to have sufficient population so that the local elderly population was large enough to provide a sample of 500 elderly subjects. Sites with known endemic diseases (including Keshan disease, Kaschin-Beck disease, goiter and Cretinism, and fluorosis) were excluded from consideration. Details on site selection process were described previously [12].

Residents aged 65 years or older from these four counties were invited to participate in the study. The only exclusion criterion is severe hearing loss which makes it impossible to conduct cognitive assessment. For each county included in the study, interviews were conducted from village to village, and completed when 500 elderly participants were enrolled. The total sample size was 2000 at baseline, 95 participants were lost during the 2.5 year follow-up because of death. The study was approved by Indiana University Institutional Review Board and the Institute for Environmental Health and Related Safety, Chinese Center for Disease Control and Prevention.

Hypertension and Blood Pressure Measurements

During the interview, participants were asked whether they were ever told by a doctor or a health care professional that they had hypertension or high blood pressure defined as systolic blood pressure $\geqslant$140 mmHg, or diastolic blood pressure $\geqslant$90 mmHg. A follow-up question of

whether they were taking any anti-hypertensive medications was asked if they answered "yes" to the hypertension question.

A physical examination was also conducted during the interview. Two consecutive measurements of systolic and diastolic blood pressure were taken by trained interviewers using a mercury sphygmomanometer while the participant was seated following the protocol outlined in the Hypertension Detection and Follow-up Program and used in other studies [13]. The average of the two blood pressure measurements was used in all analyses. The hypertension and blood pressure measures were collected at baseline, 2.5 and 7 years after baseline. In this analysis, hypertension was defined as systolic blood pressure 140 mmHg or higher, diastolic blood pressure 90 mmHg or higher, or reported use of anti-hypertensive medication.

Selenium Measures

Nail samples were collected at the time of interview and stored in clean plastic bags during the baseline survey. Fluorometric determination of trace amount of selenium with 2, 3-diaminonaphthalene was used to determine trace amounts of selenium in nails. The details on laboratory procedures and quality control measures were described elsewhere [12].

Covariates

Other information collected during the survey included age, gender, education (whether the participant had attended school), alcohol consumption and smoking history. Participants' height and weight were also measured during the interview. Body Mass Index (BMI, defined as body weight in kilograms divided by height in meters squared) was derived from height and weight measurements. The International Physical Activity Questionnaire (IPAQ) was added during the 2.5-year and 7-year follow-up survey. Physical activity was classified into the three categories of

low, moderate and high according to the Guidelines for Data Processing and Analysis of the IPAQ [14].

Statistical analysis

To capture potential non-linear relationships between selenium levels and blood pressure measures, the study population was divided into quintiles according to nail selenium levels. In addition to selenium levels, we considered the following variables potential confounding factors possibly related to both blood pressure and selenium levels: age, gender, education, BMI, smoking, alcohol consumption and IPAQ.

Analysis of variance (ANOVA) was used to compare the means of continuous variables and chi-square test was used to compare proportions of categorical variables across selenium quintile groups. Analysis of covariance (ANCOVA) models were used to determine the association between selenium level and baseline blood pressure adjusting for other covariates. Logistic regression models were used to determine the association between selenium levels and baseline hypertension adjusting for the aforementioned confounders.

Participants who were normotensive at baseline were included in the longitudinal analysis. The incidence rates of hypertension were compared among the five selenium quintile groups using chi-square test. Time to incident hypertension during the 7-year follow-up was used as the outcome variable in Cox proportional hazards regression models to examine the effect of baseline selenium level on the incidence of hypertension. Participants who remained normotensive were censored at the last evaluation.

All analyses were performed using SAS9.1 for Windows (SAS Institute Inc., Cary, North Carolina, USA). $P<0.05$ was considered statistically significant.

Results

For the baseline sample of 2000 participants, mean age was 71.9±5.6 and mean nail selenium level is 0.413±0.183μg/g. Among the five nail selenium quintile groups significant differences were seen on gender, education, alcohol consumption, smoking, BMI， systolic blood pressure, diastolic blood pressure and hypertension rate, while no difference in age was observed, as shown in Table 1. The rate of hypertension was 63.50% for the total population at baseline. Hypertension rates at baseline ranged from 43.78%, 62.22%, 66.33%, 69.52%, to 75.81% with the increasing order of selenium level in the selenium quintile groups respectively (Figure 1).

Univariate analysis indicated that the males, smokers and alcohol consumers had significantly lower selenium level compared with the females, non-smokers and non-alcohol consumers, respectively and that selenium was positively associated with BMI ($P<0.05$ for all comparisons. Results not shown). Selenium level in participants with hypertension was significantly higher than in the normotensive, with mean selenium levels 0.442±0.181μg/g and 0.364±0.175μg/g in the hypertensive and normotensive participants, respectively.

In Table 2, we present results on the association between selenium quintiles and baseline hypertension or blood pressure adjusting for various covariates. Parameter estimates from the ANCOVA models are the estimated differences in mean blood pressure measures between each selenium quintile group and the reference group while adjusting for other covariates. Comparing to participants in the lowest selenium quintile group, those in the four other quintile groups had significantly higher systolic blood pressure and diastolic blood pressure. Higher baseline selenium is also significantly associated with hypertension with odds ratios of 2.17(95%CI: 1.63 to 2.90), 2.46(95%CI: 1.83 to 3.23), 2.72(95%CI: 2.00 to 3.70), 3.55 (95%CI: 2.59 to 4.87) from the second selenium quintile to the fifth quintile, respectively. There are no differences on

hypertension rates among the four top quintile groups since the confidence intervals of the odds ratio estimates overlap.

In the 730 participants with normal blood pressure at baseline, 635 participated in the follow-up evaluations (95 had died), 360 had developed incident hypertension. Baseline population characteristics did not differ between those with incident hypertension and the normotensive with respect to age, gender, education, alcohol consumption, smoking and diastolic blood pressure (Table 3). However, selenium level, BMI and systolic blood pressure were significantly higher in those with incident hypertension than in the normotensive.

Crude incidence rates for hypertension were 45.83%, 52.27, 62.50%, 70.48%, 62.79% from the first quintile to the fifth selenium quintile groups, respectively (Figure 2). In Table 4, we present results of the Cox model that baseline selenium level was significantly associated with the incidence of hypertension adjusting for other covariates. Comparing to the lowest selenium quintile group, hazard ratios for incident hypertension were 1.41 (95%CI: 1.03 to1.94), 1.93 (95%CI: 1.40 to 2.67), 2.35 (95%CI: 1.69 to 3.26) and 1.94 (95%CI: 1.36 to 22.77) for the second selenium quintile to the fifth selenium quintile groups, respectively.

Discussion

In this study, we examined the associations between selenium level and hypertension in a 7-year follow up of elderly Chinese population in the Selenium and Cognitive Decline study. We found that participants with higher selenium levels had higher blood pressure and hypertension rate at baseline, and participants with higher baseline selenium levels also had greater risk of developing hypertension.

There were four published longitudinal studies focusing on the association between selenium and blood pressure or hypertension [8, 15-17]. In a prospective study in Belgium [8], a significant

protective effect of selenium on incident hypertension was observed in young adult males, but no significant relationship was observed in females. The other three studies reported non-significant associations between selenium and hypertension [15-17]. A previous systematic literature review published in 2014 found no conclusive evidence supporting an association between selenium levels and hypertension [18]. Our findings are consistent with results from several cross-sectional studies and case-control studies. In the US National Health and Nutrition Examination Survey data (2003–2004), high serum selenium concentrations were associated with higher prevalence of hypertension [10]. Another cross-sectional study in 3387 males aged 53-74 years also found an association between higher selenium levels and higher hypertension rates [11]. Similar result was also reported in a case-control study in France [9]. However, there were studies reporting non-significant association [19-21] while others supported an inverse association between selenium levels and hypertension [6, 22, 23]. In a cross-sectional study on 722 males with mean age 54 years old in eastern Finland higher selenium level was found to be associated with lower hypertension rate [6]. Two case-control studies reported lower selenium in hypertensive patients than in normal controls [22, 23]. In addition to observational studies, randomized controlled trials with selenium as one of the intervention agents also reported protective effect on hypertension [24, 25].

The inconsistent results from previous studies may be partially due to the difference in selenium exposure levels among the different study populations. In most previous studies, selenium's harmful effect on hypertension was more likely to be seen in selenium-replete populations and protective effect seen in selenium-deplete populations [18]. Moreover, the definition of hypertension varied among different countries, such as Belgium [8], Denmark [11], Finland [26], and the United States [17]. Other factors contributing to the inconsistent results include

differences in study design, adjustment for confounding factors and the difference in the age range covered by a study. While the biological mechanism underlying a positive association between selenium and blood pressure measure is not yet fully understood, selenium's effect on oxidative stress has been investigated in animal studies. As a key component of many antioxidant enzymes, such as glutathione peroxidase (GPx) selenium plays an important role in oxidative stress [27], which is recognized as one of the fundamental mechanisms underlying hypertension [2]. An experimental study had observed that selenium at low doses can provide significant protection of the human coronary artery endothelium against damage by oxidative stress [28]. In an animal model, dietary supplementation with selenium was associated with lower levels of cardiac oxidative damage and increased antioxidant expression, as well as a reduction in disease severity and mortality in spontaneously hypertensive rats [29]. Although experimental results mentioned above support the hypothesis that selenium deficiency is associated with oxidative stress, nonlinear dose-response relationship between selenium exposure and oxidative stress biomarkers was observed in a recent study suggesting that high selenium levels increase oxidative stress [30]. Our study, unfortunately, did not have measures of other oxidative stress markers and will not be able to examine the role of oxidative stress in the association between selenium and hypertension. More future studies on the relationship among selenium level, oxidative stress and hypertension are needed.

Thus far, most studies examining the relationship between selenium and hypertension have either included subjects over a very broad age range or have focused on middle-aged adults or children. Our study focused exclusively on the elderly population where hypertension is much more prevalent. Enrolling participants from rural areas also ensured that the majority of the

participants in our study had lived in the same village their entire lives and they were not taking dietary supplements, thus providing a stable, long-term measure of selenium exposure.

It is important to note that nail selenium represents a more stable measure of long-term exposure to selenium [31] and selenium level in nails does not fluctuate greatly with daily selenium intake in the diet [32]. In a previous study in the same cohort [12], we have confirmed that selenium levels in nail samples were significantly correlated with selenium levels measured in blood and dietary intake derived from food frequency questionnaire. Although we are unable to compare selenium levels in our study directly with selenium levels measured in blood or hair samples in other studies, the selenium level in our cohort was similar to the Finland study of age 55-69 year old sample [32], and lower than toenail selenium levels in the American [11,33] and the Northern Italy samples [34].

Strengths and limitations

Our study has a number of strengths. The first is the prospective study design and the relatively large sample size ensuring adequate statistical power. The second is the relatively low selenium level in the study population without selenium supplementation, providing an opportunity to explore the associations between selenium exposure and hypertension. The third is the selenium measurements in nail samples, which provided a relatively long-term measure of exposure compared with selenium measured in blood or urine samples.

There are also several limitations in this study. The first is that the cohort included participants older than 65 years of age. Therefore, it is not known whether the observed association between selenium and hypertension holds in populations of younger subjects. Another limitation was that glutathione peroxidase (GPx) activity was not measured. Measuring GPx levels may enable us to compare enzyme activity to selenium levels and gain a better understanding of the association

between selenium and hypertension. Finally, the current study did not consider genetic factors or other environmental factors that may influence or modify the association between selenium level and the risk of hypertension.

Conclusions: Our results, both cross-sectional and longitudinal, suggest that higher selenium may be associated with the risk of hypertension. Future studies in other populations of similar or different age composition are needed to confirm our findings. In addition, studies that incorporate more biomarkers are needed to elucidate the biological mechanism underlying the association between selenium and hypertension.

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Conflict of interest

The authors declare that there are no conflicts of interests.

Ethical standards

The authors declare that all the experiments of this study complied with the current laws of China in which they were performed.

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Table 1: Baseline characteristics of participants by nail selenium quintile groups.

Characteristics	Total (n=2000)	Quintile groups of selenium level in nail samples (μg/g)					P Value
		Q1(n=402) (≤0.233)	Q2 (n=405) (0.234-0.362)	Q3 (n=395) (0.363-0.442)	Q4(n=397) (0.443-0.552)	Q5(n=401) (>0.552)	
Nail selenium, (μg/g)	0.413±0.183	0.187±0.030	0.302±0.038	0.407±0.022	0.492±0.032	0.683±0.143	<0.0001
Age (years)	71.9±5.6	72.0±5.6	72.2±5.6	71.9±5.5	71.8±5.4	71.6±5.7	0.6858
Female (%)	53.7	52.0	45.2	47.1	59.2	64.8	<0.0001
Attended school（%）	37.7	29.9	40.5	41.3	37.5	39.2	0.0061
Alcohol consumer (%)	43.6	59.7	50.9	41.5	32.2	33.2	<0.0001
Smoker (%)	46.4	45.0	51.1	53.2	41.6	41.2	0.0007
BMI (kg/m^2)	21.94±3.51	21.05±2.70	21.07±2.99	21.78±3.56	22.61±4.08	23.19±3.55	<0.0001
Systolic BP (mm Hg)	145.7±25.0	133.8±20.8	144.4±23.0	148.6±25.8	150.1±26.6	151.6±24.4	<0.0001
Diastolic BP (mm Hg）	83.6±12.7	80.0±12.2	84.1±12.3	84.6±13.4	84.5±13.5	84.8±11.6	<0.0001
Hypertension (%)	63.50	43.78	62.22	66.33	69.52	75.81	<0.0001

Table2: Results from analysis of covariance (ANCOVA) model and logistic regression model on the association between nail selenium level, blood pressure measures and hypertension rate at baseline.

Outcome variable	Quintile groups of selenium level in nail samples (μg/g)					P Value
	Q1(n=402) (≤0.233)	Q2 (n=405) (0.234-0.362)	Q3 (n=395) (0.363-0.442)	Q4(n=397) (0.443-0.552)	Q5(n=401) (>0.552)	
	Parameter Estimates from ANCOVA Models*					
Systolic BP, mm Hg*	0.0(reference)	10.87 (7.58,14.16)	14.45 (11.09,17.81)	15.26 (11.86,18.66)	16.05 (12.65,19.45)	<0.0001
Diastolic BP, mm Hg*	0.0(reference)	3.91 (2.17,5.64)	3.88 (2.10,5.65)	3.48 (1.68,5.27)	3.66 (1.87,5.46)	<0.0001
	Odds Ratios from Logistic Regression Model**					
Hypertension/Normal**	1.0(reference)	2.17(1.63,2.90)	2.46(1.83,3.23)	2.72(2.00,3.70)	3.55(2.59,4.87)	<0.0001

Note: * ANCOVA model adjusting for age, gender, BMI, education, smoking, and alcohol consumption.

**Logistic regression model adjusting for age, gender, BMI, education, smoking, and alcohol consumption.

Table 3: Comparison of baseline characteristics between participants with incident hypertension and those normotensive during follow-up.

	Overall (n=635)	Normotensive (n=275)	Incident Hypertension (n=360)	P Value
Nail selenium, (µg/g)	0.367±0.177	0.340±0.188	0.387±0.165	0.0008
Age (years)	71.1±5.2	71.1±5.3	71.1±5.2	0.8862
Female (%)	51.97	52.36	51.67	0.8617
Attended school（%）	35.43	38.18	33.33	0.2056
Alcohol consumer (%)	44.41	47.27	42.22	0.2044
Smoker (%)	48.03	48.36	47.78	0.8836
BMI (kg/m^2)	21.05±3.07	20.62±2.71	21.39±3.28	0.0017
Systolic BP (mm Hg)	123.3±10.3	121.9±10.3	124.4±10.3	0.0018
Diastolic BP (mm Hg)	74.9±7.8	74.6±7.3	75.2±8.2	0.3209

Table 4: Hypertension incidence rate and hazard ratios for incident hypertension by selenium quintile groups during follow-up.

Selenium quintile groups	Incidence rate (%)	Hazard ratio(95% CI)	P- value
Q1 (n=192)	45.83	1.0 (reference)	--
Q2 (n=132)	52.27	1.41(1.03,1.94)	0.0331
Q3 (n=120)	62.50	1.93(1.40,2.67)	<0.0001
Q4 (n=105)	70.48	2.35(1.69,3.26)	<0.0001
Q5 (n=86)	62.79	1.94(1.36,22.77)	0.0002

Note: Cox proportional hazards regression model adjusting for age, gender, BMI, education, smoking, alcohol consumption, and physical activity.

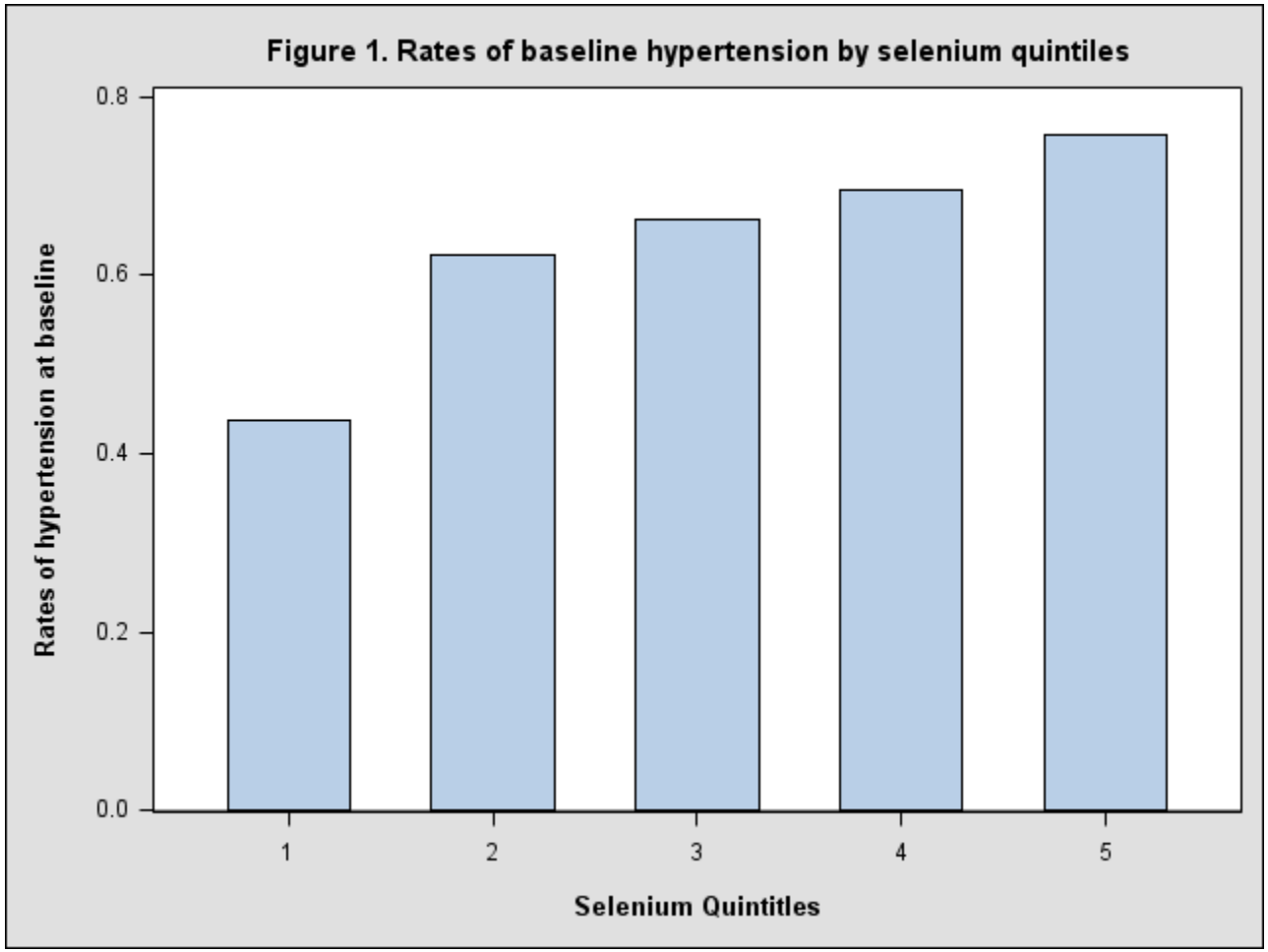
Figure 1. Rates of baseline hypertension by selenium quintiles
Rates of hypertension at baseline
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
Selenium Quintitles

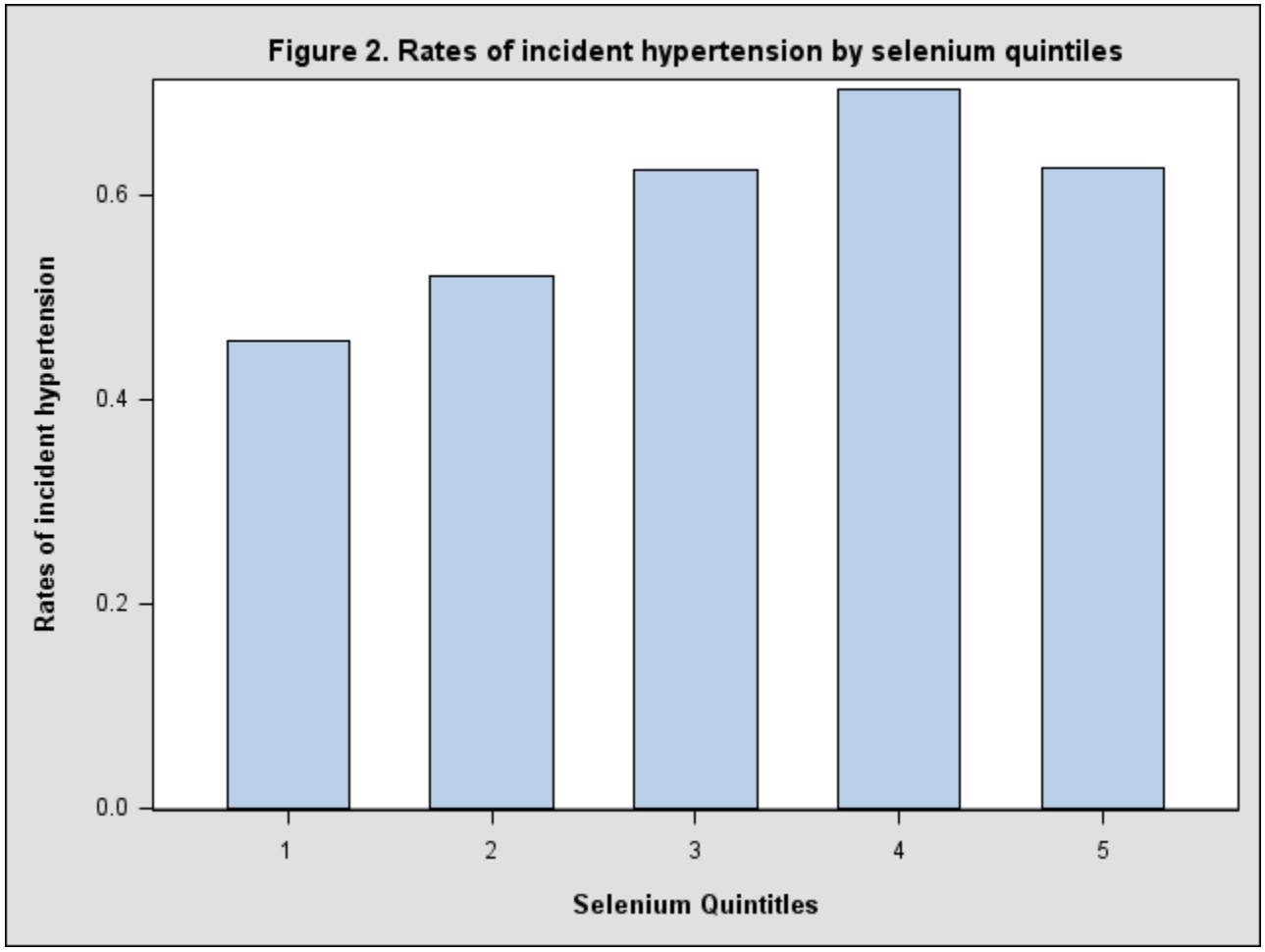
Figure 2. Rates of incident hypertension by selenium quintiles
Rates of incident hypertension
0.0
0.2
0.4
0.6
1
2
3
4
5
Selenium Quintiles