Public Health Nutrition: 12(12), 2371–2376 doi:10.1017/S1368980009005102

Selenium level is associated with *apoE ε4* in rural elderly Chinese

Sujuan Gao[1,*], Yinlong Jin[2], Kathleen S Hall[3], Chaoke Liang[2], Frederick W Unverzagt[3], Feng Ma[2], Yibin Cheng[2], Jianzhao Shen[1], Jingxiang Cao[2], Janetta Matesan[1], Ping Li[4], Jianchao Bian[5], Hugh C Hendrie[3,6,7] and Jill R Murrell[8]

[1]Department of Medicine, Indiana University School of Medicine, 410 West 10th Street – Suite 3000, Indianapolis, IN 46202-2872, USA: [2]Institute for Environmental Health and Related Product Safety, Chinese Center for Disease Control and Prevention, Beijing, People's Republic of China: [3]Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN, USA: [4]Sichuan Provincial Center for Disease Control and Prevention in China, Chengdu, People's Republic of China: [5]Shandong Institute for Prevention and Treatment of Endemic Disease in China, Jinan, People's Republic of China: [6]Indiana University Center for Aging Research, Indianapolis, IN, USA: [7]Regenstrief Institute, Inc., Indianapolis, IN, USA: [8]Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA

Submitted 8 May 2008: Accepted 7 January 2009: First published online 12 March 2009

Abstract

Objective: Se is an essential trace element in human nutrition associated with antioxidant activity. Previous studies on predictors of toenail Se or serum Se have mostly concentrated on demographic factors such as age and gender. The present paper examines the association between *apoE* genotype and Se levels in nail samples in a rural elderly Chinese cohort.

Design: Two thousand Chinese aged 65 years and over from four counties in China were enrolled in a cohort to study the association of Se with cognitive decline. Nail samples were collected from each participant and analysed for Se levels. Dietary Se intake was estimated from an FFQ using Se contents measured in food items collected from each village. Blood samples on filter cards were collected and analysed for *apoE* genotype. Mixed-effect models were constructed with nail Se level as the dependent variable and each village as the random effect, which controlled for the potential confounding effect from correlation in Se measures obtained from participants residing in the same village.

Results: In this elderly Chinese cohort, carriers of the *apoE ε4* allele had significantly lower Se levels measured in nail samples than non-carriers after adjusting for other significant covariates and controlling for estimated dietary Se intake. There was no significant difference between the two genotypes on estimated Se dietary intake ($P = 0{\cdot}6451$).

Conclusions: Future studies are needed to examine the mechanism underlying the association between the *apoE ε4* allele and Se levels, including the role of oxidative stress and that of reduced lipid metabolism in the *apoE ε4* carriers.

Keywords
Selenium
Nail samples
Dietary intake
apoE ε4

Se is an essential trace element in human nutrition associated with the activity of the antioxidant enzyme glutathione peroxidase. It is considered to be a protective agent against free radicals through enhanced enzyme activity. Associations between low Se and increased risk in various disease indices (cancer, CVD, reproduction and neonatal health, asthma) have been reported[1,2].

There is increased interest in using Se as a biomarker for various cancer outcomes[3,4]. Measuring Se intake in large cohort studies is often difficult because populations are often mobile and consume foods that were produced and prepared in different areas of the world. Se content in foods, especially grain, is highly variable depending on the Se content of the soils in which they are grown[5]. Moreover, supplements containing Se are often ingested particularly by health-conscious individuals, further confounding the results. The rural elderly Chinese population represents a unique opportunity for studying Se exposure. The rural Chinese are unusually stable with most living in the same village throughout their entire life and consuming food that is locally grown. In addition, it is rare for these villagers to take dietary supplements. Chinese scientists have assembled extensive data on Se distributions in many parts of the country; hence it is

*Corresponding author: Email sgao@iupui.edu

possible to select sites with differing Se levels so that an extended range can be achieved to maximize statistical power for detecting potential associations. We have recruited a cohort of 2000 elderly Chinese from four rural counties to study Se level and its association with various health outcomes[6].

The *apoE* gene is involved in lipoprotein metabolism and is a risk factor for Alzheimer's disease and cardiovascular disorders. There are also previous reports that an Se-deficient diet or targeted removal of a gene that causes complete loss of selenoprotein expression results in increased plasma cholesterol concentration with an increase in apoE protein[7–9]. Previous studies on predictors of toenail Se or serum Se have mostly concentrated on demographic factors such as age and gender. In the present paper, we examine the association between *apoE* genotype and Se levels measured in nail samples.

Methods

Study population

Two thousand Chinese aged 65 years and over from four counties in China were enrolled in the study. Two sites were from Sichuan Province in south-west China and the other two sites were from Shandong Province in eastern China. Since Chinese scientists have mapped Se distribution in many parts of the country, these two provinces were selected because of the varying Se levels within each province. Prior to final site selection, Chinese investigators travelled to several candidate sites collecting demographic information to ensure the local elderly population was large enough to provide a sample of 500 elderly subjects. Samples of grain (corn, rice and wheat), soil, water and nail clippings from randomly selected individuals at each candidate site were also collected and analysed for Se and other trace elements. The goal was to have two sites within one province that differed in Se levels but were similar in trace element measures and other potential confounders. Sites with known endemic diseases were excluded from consideration.

Twenty-two villages were surveyed in the present study. For each village included in the study, the Chinese investigators and a team of interviewers who were employees of provincial and county Centers for Disease Control travelled to the area, established a temporary headquarters and conducted a complete census of residents over the age 65 years in the area. They enrolled eligible residents by going door-to-door, obtaining informed consent before conducting the interview and collecting biological samples. Details of the study are published elsewhere[6]. The study was approved by the Indiana University Institutional Review Board and the Institute for Environmental Health and Related Safety, Chinese Center for Disease Control and Prevention.

Information collected during the interview include age, gender, whether the participant attended school, alcohol consumption and smoking history, history of cancer, Parkinson's disease, diabetes, hypertension, stroke, heart attack, head injury and bone fracture. Participants' height, weight and blood pressure (two times) were also measured during the interview. BMI was derived from height and weight measurements. The average of the two blood pressure measures was used in our analyses.

Se measures

Nail samples from all study subjects were collected at the time of interview and stored in clean plastic bags labelled with subject identification numbers. The method of fluorometric determination of trace amounts of Se with 2,3-diaminonaphthalene, described in detail elsewhere[10], was used to determine trace amounts of Se in the nail samples. Details on quality control measures implemented for the study have also been described previously[6].

apoE *genotype*

Blood spots on filter paper were collected from all study participants at the end of the interview. *apoE* genotype was determined by eluting DNA from a dried blood spot[11] followed by HhaI digestion of amplified products[12].

FFQ

An FFQ was administered asking participants their average daily intake of various grains, vegetables, meat, seafood, fruit, nuts, cooking oil, tea and water. The questionnaire had been developed and validated for use in Chinese populations[13–15]. Food samples were collected from various locations in each village and analysed using the same method as for the nail samples, thus providing Se measures in each food item for each village. Estimated daily Se intake was derived from the FFQ using Se levels analysed from food samples in the village in which the participants resided[16,17].

Statistical analysis

Univariate associations between demographic, lifestyle and medical history variables and Se measured in nail samples were examined using mixed-effect models with Se level as the dependent variable and each village as the random effect, which controls for the potential confounding effect from correlations in Se measures obtained from participants residing in the same village. Comparisons of demographic, lifestyle and medical characteristics between *apoE ε4* allele carriers and non-carriers were conducted using *t* tests for continuous variables and Fisher's exact tests for categorical variables. All variables that were either univariately associated with Se levels or associated with *apoE* status at the $P < 0{\cdot}10$ level were included in a multivariate mixed-effect model with Se level as the dependent variable and village as the random effect to control for potential within-village correlation. A final multivariate model was identified including those predictors significant at the 0·05 level. A separate

https://doi.org/10.1017/S1368980009005102 Published online by Cambridge University Press

mixed-effect model using estimated Se dietary intake as dependent variable was also conducted to examine the association of *apoE* status with Se dietary intake. To facilitate interpretation of parameter estimates from all mixed-effect models, we standardized all continuous variables in all models. To ensure that the association between *apoE* status and Se level was not due to existing CVD and potential treatments, we repeated the mixed-effect model by excluding those subjects with histories of heart attack and stroke. We also repeated the same mixed-effect models in male and female participants separately.

Results

Univariate mixed-effect models with Se levels measured in nail samples as dependent variable revealed that female participants, those who had attended school, abstainers from alcohol, non-current smokers, those with a history of stroke and those with a history of fracture appeared to have higher Se levels at the $P < 0{\cdot}10$ level.

We compared demographic, lifestyle and medical history factors by *apoE ε4* allele status (Table 1). Diastolic blood pressure was significantly higher in the *ε4* carriers than in the non-carriers ($P = 0{\cdot}0368$). Non-carriers had a significantly higher rate of fracture than *ε4* carriers (2·88 % *v.* 0·91 %, $P = 0{\cdot}0353$). Two other factors meeting inclusion criteria for the multivariate model were alcohol consumption ($P = 0{\cdot}0687$) and history of head injury ($P = 0{\cdot}0906$).

In Table 2, all variables significant at $P = 0{\cdot}10$ from all univariate analyses are included in a multivariate mixed-effect model to identify significant predictors of Se levels measured in nail samples. In addition, we included BMI tertile as an additional predictor in the model because it had borderline significance for Se level just below our cut-off point of $P = 0{\cdot}10$ ($P = 0{\cdot}1085$) and it allowed us to control for the potential effect from body composition. A separate mixed-effect model with estimated dietary Se intake as the dependent variable is also included in Table 2. *apoE ε4* carriers were shown to have significantly lower Se levels measured in nail samples, but they were not significantly different from non-carriers on estimated Se dietary intake ($P = 0{\cdot}6451$). Final multivariate models, using nail Se levels and including only factors significant at the 0·05 level, are presented in Table 3. Controlling for

Table 1 Comparisons of demographic, lifestyle and medical history variables between carriers and non-carriers of the *apoE ε4* allele: rural elderly (65 years and over) residents from Sichuan Province and Shandong Province, China (*n* 2000)

	apoE ε4 carriers (*n* 331)		Non-carriers (*n* 1669)		
	Mean	SD	Mean	SD	*P* value
Continuous variables					
Age (years)	72·01	5·68	71·89	5·53	0·7118
BMI (kg/m^2)	21·73	3·38	21·98	3·53	0·2440
Blood pressure (mmHg)					
Systolic	144·88	22·74	145·88	25·32	0·4738
Diastolic	84·96	13·15	83·37	12·54	0·0368
Se in nails (μg/g)	0·40	0·16	0·42	0·19	0·0801
	n	%	*n*	%	
Categorical variables					
Age 80+ years	39	11·78	191	11·44	
Male	142	42·90	785	47·03	0·1844
Ever attended school	124	37·46	629	37·69	0·9506
BMI tertile (kg/m^2)					0·9170
>22·94	112	33·84	556	33·33	
20·12–22·94	107	32·33	559	33·51	
≤20·11	112	33·84	553	33·15	
Ever drink alcohol	129	38·97	741	44·48	0·0687
Alcohol group					0·2368
Heavy (≥4 drinks/d)	18	5·44	97	5·83	
Regular (1–3 drinks/d)	63	19·03	396	23·78	
Moderate (<1 drink/d)	48	14·50	247	14·83	
Non-drinker	202	61·03	925	55·56	
Current smoker	84	25·38	443	26·54	0·6825
History of					
Cancer	1	0·30	12	0·72	0·7071
Parkinson's disease	2	0·60	16	0·96	0·7537
Diabetes	5	1·51	47	2·82	0·2539
Hypertension	55	16·62	283	16·97	0·9361
Stroke	11	3·32	51	3·06	0·7310
Heart attack	8	2·42	61	3·65	0·3230
Head injury	12	3·63	102	6·13	0·0906
Fracture	3	0·91	48	2·88	0·0353

https://doi.org/10.1017/S1368980009005102 Published online by Cambridge University Press

Table 2 Multivariate mixed-effect model to identify variables associated with selenium levels measured in nail samples (μg/g) and with estimated selenium dietary intake: rural elderly (65 years and over) residents from Sichuan Province and Shandong Province, China (*n* 2000). Village was included as a random effect to control for potential within-village correlation

	Se in nail samples (μg/g)			Estimated Se dietary intake (μg/d)		
Variable	Coefficient estimate	SE	*P* value	Coefficient estimate	SE	*P* value
Gender (male *v.* female)	−0·0231	0·0070	0·0010	0·2027	0·0383	<0·0001
Ever attended school	−0·0041	0·0064	0·5252	0·0363	0·0350	0·3003
BMI tertile (kg/m^2)						
>22·94	0·0060	0·0068	0·3834	0·1309	0·0374	0·0005
20·12–22·94	−0·0052	0·0064	0·4153	0·0423	0·0351	0·2284
≤20·11	Reference	–	–	Reference	–	–
Ever drink alcohol	−0·0017	0·0063	0·7908	0·0384	0·0345	0·2669
Current smoker	−0·0139	0·0066	0·0352	−0·0186	0·0362	0·6087
History of stroke	0·0418	0·0153	0·0064	−0·1448	0·0838	0·0842
History of fracture	0·0222	0·0153	0·1833	−0·0579	0·0917	0·5279
History of head injury	0·0070	0·0114	0·5394	−0·0220	0·0627	0·7258
Diastolic blood pressure (mmHg)	−0·0001	0·0002	0·5253	0·0016	0·0012	0·1794
Estimated dietary Se intake (μg/d)	0·3561	0·2048	0·0823	–	–	–
apoE ε4 carrier	−0·0141	0·0072	0·0488	−0·0180	0·0392	0·6451

Table 3 Final multivariate mixed-effect models including significant variables associated with selenium levels in nail samples (μg/g, standardized) in the entire sample and in a sub-sample of participants without history of heart attack or stroke: rural elderly (65 years and over) residents from Sichuan Province and Shandong Province, China (*n* 2000)

	Entire sample (*n* 2000)			Sub-sample (*n* 1875)		
Variable	Coefficient estimate	SE	*P* value	Coefficient estimate	SE	*P* value
Gender (male *v.* female)	−0·1211	0·0316	0·0001	−0·1074	0·0321	0·0008
Current smoker	−0·0810	0·0355	0·0225	−0·0897	0·0357	0·0122
History of stroke	0·2168	0·0826	0·0088	–	–	–
Standardized dietary Se intake (μg/d)	0·0406	0·0223	0·0688	0·0443	0·0226	0·0500
apoE ε4 carrier	−0·0800	0·0389	0·0396	−0·0767	0·0393	0·0512

Table 4 Multivariate mixed-effect models including significant variables associated with selenium levels in nail samples (μg/g, standardized) in male and female participants separately: rural elderly (65 years and over) residents from Sichuan Province and Shandong Province, China (*n* 2000)

	Males (*n* 927)			Females (*n* 1073)		
Variable	Coefficient estimate	SE	*P* value	Coefficient estimate	SE	*P* value
Current smoker	−0·1052	0·0431	0·0148	−0·0243	0·0655	0·7107
History of stroke	0·1039	0·1212	0·3916	0·3679	0·1140	0·0013
Standardized dietary Se intake (μg/d)	0·1014	0·0361	0·0050	0·0208	0·0285	0·4651
apoE ε4 carrier	−0·0734	0·0601	0·2219	−0·0755	0·0514	0·1416

gender, smoking status, history of stroke and estimated dietary Se intake, *apoE ε4* carriers had significantly lower Se levels measured in nail samples than non-carriers ($P = 0·0396$). The results of the final model demonstrate that given the same Se dietary intake level, the *apoE ε4* carriers had lower Se levels in their biological samples, equivalent to being two standard deviations lower on Se dietary intake. We also fitted the same model on a sub-sample of participants without a history of heart attack or stroke; the difference in Se level between the two genotype groups remained ($P = 0·0512$).

We also fitted the model in Table 3 to male and female participants separately; the results are presented in Table 4. Interestingly, dietary Se intake, while significantly related to nail Se levels in males, was not associated with nail Se levels in females. *apoE ε4* carriers had lower Se levels in their nail samples for both male and female participants. The differences between *apoE ε4* carriers and non-carriers were similar between males and females (0·0734 and 0·0755 standard deviations, respectively), although neither reached significance due to the reduced sample sizes from the model for the entire sample.

Discussion

In this rural elderly Chinese cohort, we found that *apoE ε4* carriers had significantly lower Se levels measured in nail samples than non-carriers after adjusting for other significant covariates and controlling for estimated dietary Se intake. The difference between the two genotype groups persisted at a similar magnitude when we repeated the

https://doi.org/10.1017/S1368980009005102 Published online by Cambridge University Press

analysis excluding participants with histories of heart attack and stroke.

Predictors of Se levels measured in the blood, toenails or diet have been described previously[3,4,18–20]. However, most of these studies focused on demographic variables. Our results identifying smoking and male gender as factors associated with lower Se levels are consistent with previous studies[19,20]. However, previous results on the association between increasing age and decreasing levels of Se were inconsistent[19,21], perhaps due to differences in age range in each study population. In the present cohort of participants aged 65 years and older, we did not find a significant association between age and nail Se levels. Nor did we find a significant association between alcohol consumption and Se level in this cohort, consistent with findings in previous studies[18–20].

To our knowledge, the present study is the first to examine the association between *apoE* genotype and Se levels in a large cohort of elderly participants. The *apoE* gene has been shown to play a central role in cholesterol uptake and lipid metabolism and the *ε4* allele is linked to increased risk of Alzheimer's disease and CVD. The mechanism underlying the association between the *apoE ε4* allele and Se levels is unclear, however. It is has been reported that *apoE ε2* and *apoE ε3* are more effective in maintaining neuronal health than is *apoE ε4*[22–24]. It is possible that *apoE ε4* carriers have increased levels of oxidative stress, ischaemia and inflammation and that the increased oxidative stress in turn reduces the pool of Se.

The Framingham Offspring Study proposed that *apoE ε4* may be associated with reduced antioxidant capacity based on the interaction between smoking and apoE on cardiovascular risk[25]. This mechanism is supported by *in vitro* results showing that protection of recombinant apoE against oxidation is greater in *ε4* non-carriers than in *ε4* carriers[26–28]. Results from animal studies indicate that apoE deficiency is associated with increased oxidative damage[29]. However, Se levels were not directly measured in these studies.

Another potential mechanism for the observed association between *apoE* genotype and Se levels is through reduced lipid metabolism in the *ε4* carriers. Animal experiments have demonstrated that Se deficiency can lead to increased levels of plasma cholesterol, apoE and oxidized LDL in rats and mice[30,31]. Oxidized LDL accumulates in atherosclerotic lesions in man[32] and increased LDL susceptibility to oxidation was shown in patients with hypercholesterolaemia[33], hypertension[34] and diabetes[35].

There seems to be a correlation between Se levels and metabolism of lipids and lipoproteins. Lipid metabolism may be affected by the oxidative state of the lipid molecule, on which Se would have a direct effect. A Finnish population with a higher proportion of *apoE ε4* homozygous carriers and high serum cholesterol was associated with lower CHD mortality than in other communities. The lower mortality was attributed to higher antioxidant status, in particular Se intake in this population[36]. An interactive relationship between Se, lipids and the *apoE* genotype may also be worth investigating.

Our result, showing that participants with a history of stroke had increased Se levels, was surprising given the previous report on the protective effects of Se on CHD. A few studies have reported lower Se levels in acute stroke patients compared with healthy controls[37], although others found no significant difference[38]. However, in one study, lower Se was found to be associated with stroke mortality, suggesting a potential explanation that the effects of Se on CHD in relation to mortality might leave fewer participants living with stroke[39]. This possibility will be examined further in our planned follow-up of this cohort.

The strength of the present study includes the extended range of Se distribution in the cohort, allowing the exploration of other factors, and the large cohort size for the investigation. An additional strength is that we also collected dietary data, so that the association between *apoE ε4* and Se could be examined controlling for dietary Se intake.

A limitation of the study is that the association between the *apoE ε4* allele and Se levels is found in an elderly Chinese population, which may limit the generalizability of the findings. Independent confirmation in other populations is needed. The study also lacked cholesterol and other lipid measures in the cohort, so that it is not possible to examine any potential interactive role played by the metabolism of lipids and lipoproteins.

Acknowledgements

The research was supported by NIH grants R01 AG019181, R01 AG09956 and P30 AG10133. None of the authors has any conflicts of interest. The authors contributed as follows. Conception and design: S.G., Y.J., K.S.H., C.L., F.W.U., J.R.M. and H.C.H. Acquisition of data: S.G., Y.J., K.S.H., C.L., F.W.U., J.R.M., F.M., J.M., Y.C., J.-C.B., P.L. and H.C.H. Analysis and interpretation of data: S.G., H.C.H. and J.-Z.S. Drafting of manuscript: S.G., Y.J., C.L. and H.C.H. Critical revision of manuscript for important intellectual content: S.G., Y.J., K.S.H., C.L., F.W.U., J.R.M., F.M., J.-Z.S., J.M., Y.C., J.-C.B., P.L. and H.C.H. Statistical expertise: S.G. and J.-Z.S. Obtaining funding: S.G., Y.J., K.S.H., C.L., F.W.U., J.R.M. and H.C.H. Administrative, technical or material support: Y.J., C.L., F.M., Y.C., P.L., J.-C.B. and J.M. Supervision: S.G., Y.J., C.L. and H.C.H. We thank Dr Linda Williams for helpful comments on results reported in this manuscript.

References

1. Flatt A, Pearce N, Thomson CD, Sears MR, Robinson MF & Beasley R (1990) Reduced selenium in asthmatic subjects in New Zealand. *Thorax* **45**, 95–99.
2. Rayman MP (2000) The importance of selenium to human health. *Lancet* **356**, 233–241.
3. Niskar AS, Paschal DC, Kieszak SM, Flegal KM, Bowman B, Gunter EW, Pirkle JL, Rubin C, Sampson EJ & McGeehin M

(2003) Serum selenium levels in the US population: Third National Health and Nutrition Examination Survey, 1988–1994. *Biol Trace Elem Res* **91**, 1–10.
4. Vogt TM, Ziegler RG, Patterson BH & Graubard BI (2007) Racial differences in serum selenium concentration: analysis of US population data from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* **166**, 280–288.
5. World Health Organization (1987) *Selenium. A Report of the International Programme on Chemical Safety. Environmental Health Criteria Series* no. 58. Geneva: WHO.
6. Gao S, Jin Y, Hall KS *et al.* (2007) Selenium level and cognitive function in rural elderly Chinese. *Am J Epidemiol* **165**, 955–965.
7. Stone WL, Stewart ME, Nicholas C & Pavuluri S (1986) Effects of dietary selenium and vitamin E on plasma lipoprotein cholesterol levels in male rats. *Ann Nutr Metab* **30**, 94–103.
8. Stone WL, Scott RL, Stewart EM & Kheshti A (1994) Lipoprotein alterations in the spontaneously hypertensive rat fed diets deficient in selenium and vitamin E. *Proc Soc Exp Biol Med* **206**, 130–137.
9. Sengupta A, Carlson BA, Hoffmann VJ, Gladyshev VN & Hatfield DL (2008) Loss of housekeeping selenoprotein expression in mouse liver modulates lipoprotein metabolism. *Biochem Biophys Res Commun* **365**, 446–452.
10. Li M, Cao J & Sun S (1991) Micro-fluorometric determination of trace amount of selenium in blood, hair and milk powder. *Chinese J Public Health* **10**, 306–308.
11. Yang M, Hendrie HC, Hall KS, Oluwole OS, Hodes ME & Sahota A (1996) Improved procedure for eluting DNA from dried blood spots. *Clin Chem* **42**, 1115–1116.
12. Hixson JE & Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha*I. *J Lipid Res* **31**, 545–548.
13. Chen J, Campbell TC, Li J & Peto R (1990) *Diet, Lifestyle and Mortality in China*. Oxford/Ithaca, NY/Beijing: Oxford University Press/Cornell University Press/People's Publishing House.
14. Cao J, Chen S & Sun S (1993) A comparison of methods for estimating large population dietary fluoride intake in coal burning high fluoride communities. *J Hyg Res* **22**, 52–54.
15. Zhai F, Popkin BM, Ma L, Yu W, Guo X, Jin S & Ge K (1996) The evaluation of 24-hour individual dietary recall method in China. *J Hyg Res* **25**, 51–56.
16. Cao J, Yan B & Zhang S (1996) Relations between heavy exposure of selenium-fluoride and human health. *J Hyg Res* **25**, 287–290.
17. He L (2003) Summary for method and application of food frequency survey. *J Foreign Med Sci: Hyg Sect* **30**, 368–371.
18. van den Brandt PA, Goldbohm RA, van't Veer P, Bode P, Hermus RJ & Sturmans F (1993) Predictors of toenail selenium levels in men and women. *Cancer Epidemiol Biomarkers Prev* **2**, 107–112.
19. Hunter DJ, Morris JS, Chute CG, Kushner E, Colditz GA, Stampfer MJ, Speizer FE & Willett WC (1990) Predictors of selenium concentration in human toenails. *Am J Epidemiol* **132**, 114–122.
20. Virtanen SM, van't Veer P, Kok F, Kardinaal AF & Aro A (1996) Predictors of adipose tissue tocopherol and toenail selenium levels in nine countries: the EURAMIC study. European Multicentre Case–Control Study on Antioxidants, Myocardial Infarction, and Cancer of the Breast. *Eur J Clin Nutr* **50**, 599–606.
21. Akbaraly NT, Hininger-Favier I, Carriere I, Arnaud J, Gourlet V, Roussel AM & Berr C (2007) Plasma selenium over time and cognitive decline in the elderly. *Epidemiology* **18**, 52–58.
22. Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* **240**, 622–630.
23. Mahley RW & Rall SC Jr (2000) Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* **1**, 507–537.
24. Weisgraber KH & Mahley RW (1996) Human apolipoprotein E: the Alzheimer's disease connection. *FASEB J* **10**, 1485–1494.
25. Talmud PJ, Stephens JW, Hawe E, Demissie S, Cupples LA, Hurel SJ, Humphries SE & Ordovas JM (2005) The significant increase in cardiovascular disease risk in *APOE*ε*4* carriers is evident only in men who smoke: potential relationship between reduced antioxidant status and ApoE4. *Ann Hum Genet* **69**, 613–622.
26. Smith JD, Miyata M, Poulin SE, Neveux LM & Craig WY (1998) The relationship between apolipoprotein E and serum oxidation-related variables is apolipoprotein E phenotype dependent. *Int J Clin Lab Res* **28**, 116–121.
27. Jolivalt C, Leininger-Muller B, Bertrand P, Herber R, Christen Y & Siest G (2000) Differential oxidation of apolipoprotein E isoforms and interaction with phospholipids. *Free Radic Biol Med* **28**, 129–140.
28. Miyata M & Smith JD (1996) Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and β-amyloid peptides. *Nat Genet* **14**, 55–61.
29. Shea TB, Rogers E, Ashline D, Ortiz D & Sheu MS (2002) Apolipoprotein E deficiency promotes increased oxidative stress and compensatory increases in antioxidants in brain tissue. *Free Radic Biol Med* **33**, 1115–1120.
30. Rosenblat M & Aviram M (1998) Macrophage glutathione content and glutathione peroxidase activity are inversely related to cell-mediated oxidation of LDL: *in vitro* and *in vivo* studies. *Free Radic Biol Med* **24**, 305–317.
31. Mazur A, Nassir F, Gueux E, Moundras C, Bellanger J, Grolier P, Rock E & Rayssiguier Y (1996) Diets deficient in selenium and vitamin E affect plasma lipoprotein and apolipoprotein concentrations in the rat. *Br J Nutr* **76**, 899–907.
32. Aviram M, Maor I, Keidar S, Hayek T, Oiknine J, Bar-El Y, Adler Z, Kertzman V & Milo S (1995) Lesioned low density lipoprotein in atherosclerotic apolipoprotein E-deficient transgenic mice and in humans is oxidized and aggregated. *Biochem Biophys Res Commun* **216**, 501–513.
33. Lavy A, Brook GJ, Dankner G, Ben Amotz A & Aviram M (1991) Enhanced *in vitro* oxidation of plasma lipoproteins derived from hypercholesterolemic patients. *Metabolism* **40**, 794–799.
34. Keidar S, Kaplan M, Shapira C, Brook JG & Aviram M (1994) Low density lipoprotein isolated from patients with essential hypertension exhibits increased propensity for oxidation and enhanced uptake by macrophages: a possible role for angiotensin II. *Atherosclerosis* **107**, 71–84.
35. Beaudeux JL, Guillausseau PJ, Peynet J, Flourie F, Assayag M, Tielmans D, Warnet A & Rousselet F (1995) Enhanced susceptibility of low-density lipoprotein to *in vitro* oxidation in type 1 and type 2 diabetic patients. *Clin Chim Acta* **239**, 131–141.
36. Luoma PV, Nayha S, Sikkila K & Hassi J (1995) High serum α-tocopherol, albumin, selenium and cholesterol, and low mortality from coronary heart disease in northern Finland. *J Intern Med* **237**, 49–54.
37. Zimmermann C, Winnefeld K, Streck S, Roskos M & Haberl RL (2004) Antioxidant status in acute stroke patients and patients at stroke risk. *Eur Neurol* **51**, 157–161.
38. Chang CY, Lai YC, Cheng TJ, Lau MT & Hu ML (1998) Plasma levels of antioxidant vitamins, selenium, total sulfhydryl groups and oxidative products in ischemic-stroke patients as compared to matched controls in Taiwan. *Free Radic Res* **28**, 15–24.
39. Virtamo J, Valkeila E, Alfthan G, Punsar S, Huttunen JK & Karvonen MJ (1985) Serum selenium and the risk of coronary heart disease and stroke. *Am J Epidemiol* **122**, 276–282.