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# Determinants of **serum zinc concentrations** in a population of French middle-age subjects (SU.VI.MAX cohort)

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Determinants of serum zinc concentrations

## ABSTRACT

**Background/Objectives:** Few studies have been conducted on determinants of serum zinc concentration, especially in France. The aim of this study was to investigate the relationships between serum zinc concentrations, and age, education, living area and life style in a large sample cohort of French adults.

**Subjects/Methods:** Blood samples were collected after 12h fasting in French adults (7448 women, 35 - 65-years-old and 4926 men, 45 - 65-years-old) participating to the SU.VI.MAX cohort at enrolment. Serum zinc was determined by flame atomic absorption.

**Results:** Serum zinc concentration was lower in women (mean: 12.9  $\mu\text{mol/l}$ , 95% confidence interval: 9.2-16.6  $\mu\text{mol/l}$ ) compared to men (mean: 13.4  $\mu\text{mol/l}$ , 95% confidence interval: 9.7-17.1  $\mu\text{mol/l}$ ,  $p < 0.0001$ ). Ten percents of the women exhibited serum zinc values lesser than 10.7  $\mu\text{mol/l}$  and 10% of the men exhibited serum zinc values lesser than 11.3  $\mu\text{mol/l}$ . Age was negatively associated with serum zinc only in men ( $p = 0.001$ ). In women, a positive association between smoking and serum zinc concentration ( $p = 0.0003$ ), and a negative relationship between highest education level and serum zinc concentration ( $p = 0.01$ ) were observed. With regard to geographical areas, the highest serum zinc concentrations were found in the Centre, and the lowest in the Southwest of France for both men and women. The association between serum zinc concentrations and food categories or macro-nutrient intake disappeared after stratifying by gender.

**Conclusions:** Age, gender, and geographic area seem the main determinants of serum zinc concentrations in this study.

**Keywords :** nutrition, zinc, lifestyle, smoking, alcohol, diet

## INTRODUCTION

Zinc is an essential component for the optimal function of a variety of biochemical and physiological processes (Hambidge, 2000; Cousins *et al.*, 2003; Tudor *et al.*, 2005). It is a major antioxidant trace element through zinc-induced metallothionein synthesis (Bell & Vallee, 2009), Zn-Cu superoxide dismutase, competition with iron and copper in Fenton's reaction and thiol group protection (Powell, 2000). Zinc plays important roles particularly in the physiopathology of eye (Grahn *et al.*, 2001; Ugarte & Osborne, 2001), skin (Rostan *et al.*, 2002; Lansdown *et al.*, 2007), bone (Peretz *et al.*, 2001; Palacios, 2006), brain (Takeda, 2001; Frazzini *et al.*, 2006). Zinc is also crucial for immunity (Fraker & King, 2004; Haase & Rink, 2009) and hormonal excretions. Decreases in serum zinc levels have been reported to be associated with a number of chronic diseases of older adults such as cancer (Ho, 2004; Costello *et al.*, 2005) and cardiovascular diseases (Leone *et al.*, 2006; Soinio *et al.*, 2007). With regard to France, zinc intake of healthy adults was estimated in the 1990s to be around 10.5 mg/d in men (Hercberg *et al.*, 1991; Pelus *et al.*, 1994) and 9 mg/d in women (Hercberg *et al.*, 1991); which is lower than the French recommendations [12 mg/d in men and 10 mg/d in women, (Martin, 2001)]. Few studies have been conducted on determinants of zinc status, especially in France. The SU.VI.MAX cohort gave us the opportunity of estimating the influence of various factors on serum zinc concentrations in a large cohort of French adult subjects.

## SUBJECTS AND METHODS

### Subjects

Subjects were participants in the SUPPLEMENTATION IN ANTIOXIDANT VITAMINS, AND MINERALS (SU.VI.MAX) study.

In brief, 12741 French adults subjects consisted of 7713 females aged 35-60 years ( $47.1 \pm 6.6$  yrs) and 5028 males aged 45-60 y ( $51.8 \pm 4.7$  yrs) were recruited between October 1994

and May 1995; none of the participants used vitamins or trace element supplements. Subjects were invited to participate by a multimedia campaign at the national level. A detailed description of the recruitment and design study has been published (Hercberg *et al.*, 1998; Hercberg *et al.*, 2004). All subjects gave their informed written consent to the study which has been approved by the medical ethics committee of Paris-Cochin (n°706) and the national committee for the protection of privacy and civil liberties.

The current analyses included 12374 subjects (4926 men and 7448 women) for whom serum zinc concentrations were available at baseline.

### **Biological Measurements**

At inclusion visit, blood samples were obtained from participants after 12h fasting. For zinc determination, blood was collected on trace element controlled Vacutainer® tubes (Becton Dickinson, Le Pont de Claix, France). Serum zinc concentrations were determined by flame atomic absorption spectrometry using a Perkin-Elmer 3100 model (Norwalk, CT, USA). Serum was 5 fold diluted in deionised water and the concentration was obtained using an external standard calibration curve carried out in 5% glycerol. Ready to use 1 g/l Zn (as nitrate) stock standard solution was from Merck (Darmstadt, Germany) and was diluted to obtain 1, 2, 4 and 6 µmol/l working standard zinc solutions. Seronorm® trace element serum was used as internal quality control (Sero®, Billingstad, Norway). In addition, the laboratory took part in two interlaboratory comparison trials organised by the French Society for Clinical Biology (SFBC, Nancy, France) and the “Centre de Toxicologie du Québec” (Sainte-Foy, Québec, Canada).

### **Dietary assessment**

Data on dietary intake were available for a subsample of 6413 subjects who reported at least six 24-h dietary records during the first 18 months of the study. The record days (two weekend days and four week days) were randomly assigned to volunteers and covered all

seasons. Collection of dietary data has been described previously ([Le Moullec et al., 1996](#); [Hercberg et al., 1998](#)). Subjects were assisted for coding foods, including photographs for estimations of portion size validated on 780 subjects in a pilot study (Le Moullec et al., 1996). Data were also collected on cooking methods, seasoning, type of foods (i.e. fresh, frozen, canned), and place and time of food intake.

The macro-nutrient intake was estimated using a computerized version of French food composition table (Feinberg et al., 1991) complemented by data based on the McCance and Widdowson's food composition table (Paul & Southgate, 1978). All dietary values reported here were based on the average intakes for six 24-h dietary records for each subject.

### **Anthropometric, socio-demographic and lifestyle data**

Information recorded at baseline included age (categorized in 6 classes: <40 years, 40 to <45 years, 45 to <50 years, 50 to <55 years, 55 to <60 years and  $\geq 60$  years with the first class including only women), gender, educational level (coded as three categories according to the highest degree obtained: primary school, high school, university or equivalent). Five categories of smoking habits were defined: never smokers, former smokers, moderate current smokers (less than 10 cigarettes per day), heavy current smokers (more than 20 cigarettes per day), and current smokers if the number of cigarettes smoked per day varied from 10 to 20. Physical activity was also divided into three categories (irregular physical activity, <1h of walking per day,  $\geq 1$ h of walking per day). Baseline height and weight were measured and body mass index (BMI) was calculated.

France was divided into 8 geographic regions ([Alps - Rhone Valley and Auvergne](#), [Centre-east](#), Mediterranean coast, Northeast, Northwest, Paris outskirts, Southwest, and West). The place of residence was defined by the zip code of each subject at baseline. Four categories were defined according to the definition of the French National Institute of Statistics and Economic Studies (Institut National de la Statistique et des Etudes Economiques, I.N.S.E.E., Paris, France) based on the economic activity of the area corresponding to each subject's zip code (Le Jeannic & Vidalenc, 1997): urban municipalities provided at least 5000 jobs;

suburbs were defined as municipalities surrounding an urban pole; mixed municipalities were those located outside an urban area, but were at least 40% of the resident population worked in an urban area; and rural municipalities consisted of all other zip codes or municipalities.

### **Statistical analysis**

Data were processed on an alpha-VMC system, and a specific database was developed using the Statistical Analysis System version 8.2 (SAS Inc, Cary, NC, USA). Serum zinc values were expressed as mean  $\pm$  standard error to the mean. Student's t-test was used to compare serum zinc concentrations and dietary intakes in men and women. Crude associations between serum zinc levels and dietary intakes were evaluated by linear regressions on the whole population. Further analyses were performed in men and women separately. After eliminating pregnant women (n=19) and oral contraceptives users (n=914), the remaining results were used to defined lower cut-offs (2.5<sup>th</sup> percentiles) for the assessment of marginal zinc deficiency. The mean levels of serum zinc were compared across categories of age, body mass index, physical activity, educational level, geographic region and smoking habits using the Fisher's test and/or a test for a linear trend across categories with various adjustments. Multiple linear regressions were performed for evaluating the association between serum zinc concentrations and albumin, dietary or food intakes after various adjustments. P < 0.05 was considered significant.

### **RESULTS**

Serum zinc concentration was significantly lower in women who did not take contraceptive pills and were non-pregnant (mean: 12.9  $\mu$ mol/l, 95% confidence interval: 9.2-16.6  $\mu$ mol/l, n=6515 compared to men (mean: 13.4  $\mu$ mol/l, 95% confidence interval: 9.7-17.1  $\mu$ mol/l, n=4926; p<0.0001). In this population with serum albumin concentrations in the normal range (37-55 g/l), serum zinc concentrations were positively associated with serum albumin levels and the relationship remained after adjustment for age, education, smoking and geographic

areas (in men  $\beta=0.156 \pm 0.035$ ,  $p<0.0001$ ; in women  $\beta=0.151 \pm 0.041$ ,  $p=0.0003$ ). Ten per cent of women had serum zinc concentrations below  $10.7 \mu\text{mol/l}$  and 10% of men had serum zinc concentrations below  $11.3 \mu\text{mol/l}$ , which are the cut-off levels for zinc deficiency recommended by IZiNCG (Hotz *et al.*, 2003; Brown *et al.*, 2004). The lower cut-offs calculated from SU.VI.MAX data varied from  $9.8$  to  $10.2 \mu\text{mol/l}$  in men and from  $9.3$  to  $10.1$  in women who did not take contraceptive pills and were non-pregnant (figure 1). A significant linear trend towards lower serum zinc concentration with age was observed only for men (figure 1). This tendency was confirmed after adjustment for smoking, education, and geographic areas ( $p<0.001$ ). Non-menopausal women taking contraceptive pills ( $12.6 \pm 0.06 \mu\text{mol/l}$ ,  $n=914$ ) exhibited significant ( $p=0.02$ ) lower plasma Zn concentration than those who did not ( $12.8 \pm 0.02 \mu\text{mol/l}$ ,  $n=4347$ ). This difference remained after adjustment for age, smoking, education, and geographical areas ( $p=0.01$ ). In contrast, serum Zn concentration was significantly higher in post-menopausal women ( $12.9 \pm 0.06 \mu\text{mol/l}$ ,  $n=2020$ ) compared to non-menopausal ones ( $12.8 \pm 0.03 \mu\text{mol/l}$ ,  $n=5380$ ,  $p=0.01$ ). The significance remains after adjustment for age, smoking, education, and geographic areas ( $p=0.05$ ). No effect of hormone replacement therapy (HRT) was observed on plasma Zn concentrations ( $12.9 \pm 0.07 \mu\text{mol/l}$  in the 789 HRT users *versus*  $13.0 \pm 0.05 \mu\text{mol/l}$  in the 1231 non users,  $p=0.12$ ). Linear trends observed in women but not in men showed decreasing serum zinc concentration with education, whereas serum zinc concentration increased with smoking (table 1). BMI and physical activity had no significant effect on serum zinc concentrations (table 1).

Living in rural, mixed, suburbs or urban areas had no effect both in men and women groups. Average values varied from  $13.3$  to  $13.6 \mu\text{mol/l}$  in men and from  $12.8$  to  $12.9 \mu\text{mol/l}$  in women depending on subgroups ( $p>0.10$ ). However, significant differences in serum zinc levels were observed according to geographic areas ( $p<0.0001$ ) both in men and women. Average values varied from  $13.2$  to  $13.9 \mu\text{mol/l}$  in men and from  $12.4$  to  $13.2 \mu\text{mol/l}$  in women. The highest serum zinc concentrations were found in the Centre-East ( $13.9 \pm 0.10$



$\mu\text{mol/l}$ ,  $n=384$  in men;  $13.2 \pm 0.08 \mu\text{mol/l}$ ,  $n= 603$  in women), and the lowest in Southwest ( $13.2 \pm 0.08 \mu\text{mol/l}$ ,  $n=490$  in men;  $12.4 \pm 0.07 \mu\text{mol/l}$ ,  $n= 669$  in women).

In the group who completed the food questionnaires, dietary intake was significantly lower in women (table 2). Daily consumption of meat, fish and cereals was higher in men than in women whereas the consumption of eggs, dairy products and fruits and vegetables were similar (table 3). Positive crude associations between serum zinc concentrations and macronutrients intakes ( $\beta= 0.00029 \pm 0.00004$  for energy;  $\beta=0.00875 \pm 0.00101$  for proteins;  $\beta=0.00493 \pm 0.00088$  for lipids;  $\beta= 0.00175 \pm 0.00035$  for carbohydrates,  $\beta=0.00990 \pm 0.00359$  for fibers,  $\beta= 0.00665 \pm 0.00113$  for alcohol,  $p<0.01$ ), red meat and giblet consumption ( $\beta=0.00073 \pm 0.00028$ ,  $p=0.01$ ), fish and sea foods ( $\beta=0.00058 \pm 0.00030$ ,  $p=0.05$ ) and bread, cereals and starchy foods ( $\beta=0.00042 \pm 0.00012$ ,  $p=0.001$ ) were observed in the whole population but disappeared after adjustment for (data not shown) or stratification by gender (tables 4 and 5).

## DISCUSSION

This study is the first large-scale study that provides information on serum zinc levels and their determinants in a national sample of French adults. Different approaches have been used to evaluate zinc status such as plasma, serum, leucocytes, red blood cells, urine, hair and saliva zinc level determination associated or not with zinc intakes and functional indices such as alpha-2 macroglobulin, thymulin, metallothioneins, activity of zinc-dependant enzymes such as carboanhydrase or alkaline phosphatase or more recently Zn transporter or metallothionein RNA (Prasad, 1985; Gibson *et al.*, 2008; Lowe *et al.*, 2009). Plasma or serum zinc levels are under tight homeostatic control and very sensitive to inflammation, cytokines and hormones and they therefore would not systematically reflect changes in zinc intakes and status (Brown *et al.*, 2004). However, plasma or serum zinc determination remain the most informative and easy to use index in large scale study such as SU.VI.MAX

taking into account both analytical and physiological criteria (Pilch & Senti, 1985; Hess *et al.*, 2007).

So far, few large scale studies have been conducted on the determinants of serum zinc concentrations. In contrast, the numerous studies performed on a limited number of participants conducted to discrepant conclusions. As serum zinc inter-individual variability is higher than 15% (Giles *et al.*, 1994; Hashim *et al.*, 1996; Ricos *et al.*, 1999), these studies must be interpreted with caution. In the present work, the large SU.VI.MAX cohort, which is a general French population not selected on specific risk factors (Hercberg *et al.*, 2004), should permit a better analysis.

In SU.VI.MAX, the average serum zinc concentrations of men and women were close to those obtained from the Second National Health and Nutrition Examination Survey (NHANES) in the United States (US) (Mares-Perlman *et al.*, 1995; 2002; Hotz *et al.*, 2003) and from European studies in [middle-age and/or](#) senior healthy subjects (Andriollo-Sanchez *et al.*, 2005; Marcellini *et al.*, 2006). Our results show that serum zinc concentration is significantly lower in women compared to men. [We were not able to analyse our data according to IZINCG as in the SU.VI.MAX study, we did not collect all the recommended conditions i.e. white blood cell count, lactating women, hormonal therapy \(Brown \*et al.\*, 2004\). Nonetheless, our results suggest that the lower cut-offs for defining zinc sub-deficiency in France are under those found in the United states \[10.1  \$\mu\text{mol/l}\$  in adult French men and 9.3  \$\mu\text{mol/l}\$  in non-pregnant French women \*versus\* 11.3  \$\mu\text{mol/l}\$  and 10.7  \$\mu\text{mol/l}\$  respectively in adult American men and non-pregnant women \(Hotz \*et al.\*, 2003\)\]. Due to the low number of pregnant women, it was not possible to calculate cut-offs for this population.](#) With regard to age, we observed differences between genders with a moderate decrease with age in men. These results were similar to those reported in the NHANES II (Hotz *et al.*, 2003). Serum zinc concentration significantly decreased in fasting US men after 60 years whereas a tendency to increase was noted from 40 to 65 years in fasting US women. These variations remained moderate as previously described in free-living population (Savarino *et al.*, 2001; Andriollo-Sanchez *et al.*, 2005) in contrast to the dramatic decrease in serum zinc

concentration observed in institutionalized elderly (Pepersack *et al.*, 2001). The lower values in non-menopausal women taking oral contraceptive was also confirmed (Hotz *et al.*, 2003; Akhter *et al.*, 2005; Fallah *et al.*, 2009) which suggests that gender differences could be partly attributed to hormonal status and/or contraceptive steroids intake. Indeed, serum zinc concentrations vary during menstrual cycle with the lowest levels during ovulatory and luteal phases and the highest during menses (Deuster *et al.*, 1987; Das & Chowdhury, 1997). The administration of estrogens has been reported to modify zinc homeostasis in animal model with a decrease in serum zinc concentrations and an increase in liver zinc uptake (Lei *et al.*, 1976). The higher serum zinc concentrations in menopausal women than in non-menopausal women confirm the influence of sex hormones on zinc concentrations. Nevertheless, this explanation remained insufficient as no difference was observed between the HRT menopausal women users and non-users as previously reported in some studies (Herzberg *et al.*, 1996; Bureau *et al.*, 2002) and despite the reduced urinary zinc excretion reported in women taking HRT (Herzberg *et al.*, 1996). Nonetheless, the influence of hormonal treatments on serum zinc concentration depends on the dose, may also reflect metabolic changes such as glucose intolerance and dyslipidemia. Differences in diet consumption could also contribute to the lower serum zinc concentrations in women than in men. Indeed, energy and macronutrient intakes were significantly lower in women compared to men. Food profile also showed gender differences as previously reported (Uitenbroek *et al.*, 1996; Wirfalt & Jeffery, 1997; Roos *et al.*, 1998; Vandevijvere *et al.*, 2009) and indicated that sources of zinc such as meat, fish and seafood were more consumed by men than by women. It has also been suggested that differences in body mass index (BMI) could explain the lower serum zinc concentrations in women than in men. Although BMI was higher in men than in women, our results did not evidence any differences in serum zinc concentration according to BMI in agreement with some previous reports (Hashim *et al.*, 1996; Benes *et al.*, 2005; Song *et al.*, 2007).

Our results highlighted smoking and education as other significant determinants of serum zinc levels only in women. The higher serum zinc concentration in heavy female smokers

compared to never and former smokers was unexpected although this observation was in agreement with some previous reports (Dubick & Keen, 1991; Faruque *et al.*, 1995; Kim *et al.*, 2003). Education is the most commonly used measure of socio-economic status in epidemiological studies (Winkleby *et al.*, 1992) and our results evidenced a serum zinc decreased with increased education. The effects of smoking on serum zinc concentration in women may involve numerous factors including dietary patterns, nutritional status, inflammation and oxidative stress (Dubick & Keen, 1991; Kim *et al.*, 2003) whereas education may influence food and alcohol consumptions, smoking habits and physical activity (Uitenbroek *et al.*, 1996; Gibson *et al.*, 2008). Dietary and food intakes as well as physical activity do not seem major factors as multiple regression analysis did not show any association between macronutrient intake or food consumption and serum zinc concentrations after stratifying by gender and no association was found between zinc and physical activity. Although in the normal range, serum albumin concentrations, the major zinc protein binding in serum is positively related to serum zinc concentrations. Other biological substances, not determined in SU.VI.MAX, are known to modify zinc homeostasis. Serum zinc concentration is very sensitive to inflammation and particularly to the pro-inflammatory cytokines IL-6 and TNF as well as by metallothioneins (Vasto *et al.*, 2007). In addition, zinc enhances translocation of NF- $\kappa$ B, a transcription factor involves in the expression of cytokines (Vasto *et al.*, 2007).

Intriguingly, we observed significant regional differences in both gender. These significant although small regional differences remain unclear. Results from the EPIC study (Slimani *et al.*, 2002) indicate wide differences in dietary patterns across Europe. In France, the dietary patterns were heterogeneous with the highest consumption of fish in the Northwest, the highest consumption of meat in the Northeast and the lowest alcohol consumption in the South (Slimani *et al.*, 2002). However, the lack of association between food consumption or macronutrient intake and serum zinc concentrations after stratification by gender suggests that the dietary intake variability within gender is insufficient to explain these associations. Nonetheless, regional differences in dietary patterns including seasoning, may modulate zinc

bioavailability due to the complex combination of nutrients and non-nutritive compounds present in diet that interact with zinc (Lonnerdal, 2000; Martin, 2001; Winichagoon, 2008). Regional differences have also been reported in European countries (Kvicala & Jiranek, 1999; Diaz Romero *et al.*, 2002). However, the positive association between zinc concentrations and volcanic soils, proposed as an explanation in Canarian islands (Diaz Romero *et al.*, 2002) was not observed in SU.VI.MAX.

In conclusion, the present work provides information from the first large-scale study conducted in French adults despite several limitations. First, dietary intakes are not available for the entire population. Second, due to the large number of participants, small differences are statistically significant as usually in large epidemiological studies. Third, the interpretation of the association between serum zinc concentrations and the studied factors remains complex as serum zinc is sensitive to a variety of factors that have not been determined in SU.VI.MAX. However, serum zinc concentrations do not seem closely related to diet which confirms the limited efficacy of this indicator for the evaluation of zinc status and that serum zinc concentrations are highly regulated in general population as previously reported in the United States (Giles *et al.*, 1994; Hashim *et al.*, 1996). We reported significant differences between men and women suggesting that serum zinc concentrations in women are more sensitive to smoking and education-related modifications than in men. Age, gender, and geographic area seem the main determinants of serum zinc concentrations. Association between serum albumin and zinc concentrations is well known (Brown *et al.*, 2004) and confirmed in this study, despite the albumin concentrations were in the normal range. For geographical variations, it would be worthy to perform complementary studies in order to identify the factors explaining these variations.

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### **Conflict of interest**

The authors declare no conflict of interest.

The authors had full access to all of the data in the study. They take responsibility for the integrity of the data and the accuracy of the data analysis.

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**Figure 1: Median and 2.5<sup>th</sup> percentiles of serum zinc (in  $\mu\text{mol/l}$ ) in SU.VI.MAX population according to age and gender**

- 2.5<sup>th</sup> percentile of serum zinc for men
- Median of serum zinc for men
- 2.5<sup>th</sup> percentile of serum zinc for non-pregnant women who did not use contraceptive pills.
- Median of serum zinc for non-pregnant women who did not use contraceptive pills

Symbols represent the mid-point for five-year age intervals (35-39y: 787 women; 40-44y: 1383 women and 116 men; 45-49y: 1849 women and 1794 men; 50-54y: 1273 women and 1493 men; 55-59y: 987 women and 1204 men; 60-65y: 236 women and 319 men). Curves were fitted using a 4<sup>th</sup> order polynomial function for age in years.

**Table 1: Serum zinc concentrations, expressed as mean  $\pm$  SEM in  $\mu\text{mol/l}$ , according to lifestyle and socio-demographic factors.**

	Factors	Men (n)	Women (n)
BMI <sup>1</sup> (kg/m <sup>2</sup> )	Normal (< 25)	13.5 $\pm$ 0.04 (2389)	12.8 $\pm$ 0.03 (5731)
	Overweight (25 $\leq$ 30)	13.5 $\pm$ 0.04 (2078)	13.0 $\pm$ 0.06 (1131)
	Obese ( $\geq$ 30)	13.5 $\pm$ 0.11 (290)	12.9 $\pm$ 0.10 (339)
	P for trend <sup>2</sup>	0.622	0.174
	P for trend <sup>3</sup>	0.831	0.287
Physical activity	Irregular	13.5 $\pm$ 0.06 (1133)	12.8 $\pm$ 0.04 (1919)
	< 1h/d walking	13.5 $\pm$ 0.05 (1251)	12.8 $\pm$ 0.04 (2577)
	$\geq$ 1h/d walking	13.5 $\pm$ 0.04 (2472)	12.9 $\pm$ 0.04 (2836)
	P for trend <sup>2</sup>	0.484	0.688
	P for trend <sup>3</sup>	0.646	0.508
Education	No or primary	13.5 $\pm$ 0.05 (1177)	13.0 $\pm$ 0.05 (1419)
	Secondary	13.6 $\pm$ 0.05 (1738)	12.8 $\pm$ 0.04 (2911)
	High school or University	13.5 $\pm$ 0.04 (1950)	12.8 $\pm$ 0.03 (3006)
	P for trend <sup>2</sup>	0.802	0.011
	P for trend <sup>4</sup>	0.952	0.018
Smoking habits	Never	13.5 $\pm$ 0.05 (1624)	12.8 $\pm$ 0.03 (3937)
	Former	13.5 $\pm$ 0.04 (2399)	12.8 $\pm$ 0.04 (2091)
	Moderate (< 10 cig/d)	13.5 $\pm$ 0.10 (333)	12.9 $\pm$ 0.08 (617)
	Smokers (10-20 cig/d)	13.5 $\pm$ 0.11 (263)	13.0 $\pm$ 0.09 (406)
	Heavy (> 20 cig/d)	13.6 $\pm$ 0.15 (144)	13.3 $\pm$ 0.15 (160)
	P for trend <sup>2</sup>	0.270	0.0003
	P for trend <sup>5</sup>	0.467	0.0003

<sup>1</sup>Body mass index: BMI

<sup>2</sup>Without adjustment

<sup>3</sup>Adjustment for age, smoking, education and geographic areas.

<sup>4</sup>Adjustment for age, smoking and geographic areas.

<sup>5</sup>Adjustment for age, education and geographic areas.

**Table 2** Macronutrient intakes in SU.VI.MAX population, expressed as mean  $\pm$  SEM.

	Men (n =2721)	Women (n=3692)	P-value
	mean $\pm$ SEM	mean $\pm$ SEM	
Energy intake (MJ/d)	10.38 $\pm$ 0.04	7.73 $\pm$ 0.03	<.0001
Carbohydrate intake <sup>1</sup> (g/d)	240.50 $\pm$ 0.83	185.61 $\pm$ 0.48	<.0001
Lipid intake <sup>1</sup> (g/d)	101.03 $\pm$ 0.26	79.73 $\pm$ 0.16	<.0001
Protein intake <sup>1</sup> (g/d)	100.75 $\pm$ 0.25	77.48 $\pm$ 0.18	<.0001
Fiber intake (g/d)	21.25 $\pm$ 0.11	17.47 $\pm$ 0.07	<.0001
Alcohol intake <sup>1</sup> (g/d)	29.39 $\pm$ 0.43	10.94 $\pm$ 0.21	<.0001

<sup>1</sup>Adjustment for total energy intake.

**Table 3: Food intakes in SU.VI.MAX population, expressed as mean  $\pm$  SEM in g/d.**

	Men (n =2721)	Women (n=3692)	P-value
	mean $\pm$ SEM	mean $\pm$ SEM	
Red meat and giblets	68.01 $\pm$ 1.78	48.74 $\pm$ 1.23	<.0001
White meat and poultry	32.18 $\pm$ 1.43	21.05 $\pm$ 0.82	<.0001
Eggs	20.26 $\pm$ 1.01	18.58 $\pm$ 0.80	0.18
Fish and sea foods	46.65 $\pm$ 1.61	36.65 $\pm$ 1.19	<.0001
Dairy products and cheese	259.65 $\pm$ 4.17	262.88 $\pm$ 3.68	0.56
Fruits and vegetables	299.04 $\pm$ 4.89	290.22 $\pm$ 3.86	0.15
Bread, cereals and starchy foods	298.95 $\pm$ 4.18	200.94 $\pm$ 2.68	<.0001



**Table 4: Multiple linear regression between serum zinc and macronutrient intake.**

	Serum zinc concentration						
	Model	Men			Women		
		$\beta^1$	SE <sup>1</sup>	p-value	$\beta^1$	SE <sup>1</sup>	p-value
Energy intake, kcal/d	1	0.000005	0.00006	0.94	-0.00004	0.00007	0.59
	2	-0.000007	0.00006	0.91	-0.00002	0.00007	0.75
Protein intake, g/d	1	0.00244	0.00160	0.13	0.00158	0.00165	0.34
	2	0.00190	0.00160	0.24	0.00160	0.00170	0.35
Lipid intake, g/d	1	0.00198	0.00136	0.14	-0.00217	0.00132	0.10
	2	0.00150	0.00140	0.27	-0.00170	0.00140	0.23
Carbohydrate intake, g/d	1	-0.00049	0.00050	0.33	-0.00005	0.00056	0.93
	2	-0.00050	0.00050	0.31	0.00020	0.00060	0.77
Fiber intake, g/d	1	-0.00843	0.00503	0.09	-0.00169	0.00541	0.76
	2	-0.00690	0.00520	0.18	0.00070	0.00560	0.89
Alcohol intake, g/d	1	-0.00144	0.00148	0.33	0.00105	0.00232	0.65
	2	-0.00130	0.00150	0.40	-0.00070	0.00240	0.76

<sup>1</sup>Results of linear regression were expressed by linear regression coefficient ( $\beta$ ) and standard error (SE).

Model 1: Without adjustment

Model 2: Adjusted for age, education, smoking, geographic area and all factors in the table,

**Table 5: Multiple linear regression between serum zinc and food intakes.**

	Serum zinc concentration						
	Model	Men			Women		
		$\beta^1$	SE <sup>1</sup>	p-value	$\beta^1$	SE <sup>1</sup>	p-value
Red meat and giblets	1	0.00016	0.00038	0.68	0.00041	0.00040	0.31
	2	0.00027	0.00039	0.48	0.00027	0.00041	0.51
White meat and poultry	1	-0.00036	0.00047	0.45	0.00051	0.00061	0.40
	2	-0.00034	0.00048	0.48	0.00039	0.00062	0.53
Eggs	1	-0.00015	0.00068	0.82	0.00038	0.00062	0.54
	2	-0.00011	0.00068	0.87	0.00046	0.00063	0.47
Fish and sea foods	1	0.00014	0.00042	0.75	0.00048	0.00042	0.25
	2	0.00010	0.00043	0.81	0.00051	0.00042	0.23
Dairy products and cheese	1	0.00030	0.00016	0.07	0.00002	0.00014	0.86
	2	0.00028	0.00016	0.09	0.00007	0.00014	0.62
Fruits and vegetables	1	-0.00018	0.00014	0.19	-0.00005	0.00013	0.69
	2	-0.00017	0.00014	0.23	-0.00003	0.00013	0.83
Bread, cereals and starchy foods	1	-0.00006	0.00016	0.70	0.00005	0.00019	0.78
	2	-0.00012	0.00016	0.47	0.00010	0.00019	0.58

<sup>1</sup>Results of linear regression were expressed by linear regression coefficient ( $\beta$ ) and standard error (SE).

**Model 1:** Without adjustment

**Model 2:** Adjusted for age, education, smoking, geographic area and all factors in the table,

