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Effect of zinc supplementation on *in vitro* copper-induced oxidation of low-density lipoproteins in healthy French subjects aged 55–70 years: the Zenith Study

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Zn has been shown to possess antioxidant properties *in vitro* and *in vivo*. As inadequate dietary Zn intake has been reported in these populations, Zn supplementation may protect against oxidative stress and thereby limit the progression of degenerative diseases in such populations. We conducted the present study to evaluate the long-term supplementation effects of two moderate doses of Zn on *in vitro* Cu-induced LDL oxidation in French men and women. Three groups of sixteen healthy subjects aged 55–70 years from each sex participated in this randomized double-blind, placebo-controlled study. Each group received for six months either 0, 15 or 30 mg supplemental Zn per d. At the beginning and at the end of the supplementation periods, dietary intakes of Zn, Cu, Fe and vitamin E were estimated using 4 d food-intake records (including the weekend) and the GENI program. Zn, Cu, Fe and vitamin E status were also determined. *In vitro* LDL oxidizability (basal conjugated diene level, maximal conjugated diene formation and lag time) and lipid parameters were also determined. Dietary intakes of Zn, Cu, Fe and vitamin E were adequate in this population. Zn supplementation significantly increased serum Zn levels but did not significantly modify Cu, Fe or vitamin E status. However, Zn supplementation had no effect on *in vitro* LDL oxidation parameters, nor were there any sex-related differences in *in vitro* LDL oxidizability. The present study showed that long-term Zn supplementation of healthy subjects aged 55–70 years had no effect on *in vitro* Cu-induced LDL oxidation under the study conditions.

Low-density lipoprotein: Oxidation: Zinc status: Zinc supplementation: Human subjects

The involvement of free radical oxygen species in the general ageing process has been well documented, and an enhanced oxidative stress in elderly people has been reported to increase the incidence of pathologies such as CVD, cancer and diabetes (Harman, 1992). Uncontrolled free radical production in ageing is considered to result from both an enhanced production of reactive oxygen species, especially through the mitochondrial pathway (Balaban *et al.* 2005), and through lowered antioxidant defences. Several studies have demonstrated that endogenous antioxidant defences and antioxidant enzyme activities decline with age (Napoli *et al.* 1997; Jones *et al.* 2002; Maggio *et al.* 2003) while nutritional antioxidant intakes decrease (Vaquero, 2002). Low intakes of antioxidant nutrients such as vitamins and trace elements such as Zn might, therefore, play an important role in atherogenesis (Reaven *et al.* 1999).

The antioxidant properties of Zn have been demonstrated both *in vitro* and *in vivo* and several mechanisms have been advanced. These mechanisms include, in particular, the protection of protein sulphydryl groups against oxidation and the direct inhibition of the production of reactive oxygen species by transition metals (Bray & Bettger, 1990; Powell, 2000). Another possible antioxidant action of Zn could be related to protection against vitamin E restriction, the contribution to the structure of the antioxidant enzyme Zn,Cu-superoxide dismutase, and the maintenance of tissue concentrations of metallothionein, a powerful free radical scavenger (DiSilvestro, 2000). Zn was also shown to decrease *in vitro*

Abbreviation: TBARS, thiobarbituric acid reactive substances.

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Fe-dependent hydroxy radical production (Coudray *et al.* 1993). Such functions might act to prevent or attenuate the oxidation of biological molecules, in particular the lipid components. Therefore, Zn supplementation may be beneficial against oxidative stress and may directly or indirectly modify *in vivo* LDL oxidizability, thereby counteracting the atherosclerotic process (Gatto & Samman, 1995; Hennig *et al.* 1996; Beattie & Kwun, 2004). Zn is also known to modify lipid metabolism (Hiller *et al.* 1995; Schlegel-Zawadzka *et al.* 2004), and thus, by changing the LDL fatty acid composition, Zn could indirectly modify *in vivo* LDL oxidizability. Although some studies have already demonstrated that Zn supplementation may improve endothelial cell functions (McClain *et al.* 1995) and play a role in the prevention of CVD (Hercberg *et al.* 1998), there are still very few studies that have investigated the effect of Zn supplementation on LDL oxidation in man (Gatto & Samman, 1995).

The present study aimed to investigate, in French subjects aged 55–70 years, the potential beneficial effect on the time-course of *in vitro* LDL oxidation of long-term Zn supplementation using two moderate doses of Zn, corresponding to about one-and-a-half and three times the French RDA for Zn which are set at 9·5 mg/d for women and at 11·5 mg/d for men (Arnaud, 2001). Moreover, because of the close link between Zn and other nutritional antioxidant or pro-oxidant micronutrients such as Cu, Fe and vitamin E, and their possible impact on *in vivo* LDL oxidizability, the dietary intake and the status of these micronutrients were also determined in the present study.

Subjects and methods

Study design

The study design was a randomized, double-blind, placebo-controlled intervention trial in men and women aged 55–70 years. Subjects were randomly assigned to one of three groups to receive either a placebo, or 15 or 30 mg Zn/d as two capsules in the morning for 6 months. The supplemental Zn was given as Zn gluconate, which was prepared and supplied by E-Pharma (Creapharm, Gannat, France). The placebo capsule contained 199 mg lactose and 1 mg magnesium stearate. The 7·5 mg Zn capsule contained 56·9 mg Zn gluconate, 142·1 mg lactose and 1 mg magnesium stearate. The 15 mg Zn capsule contained 113·7 mg Zn gluconate, 85·3 mg lactose and 1 mg magnesium stearate. The capsules of the three batches were identical in their shape, colour and weight. Capsules were distributed to the subjects at the beginning of the trial and at 3 months. At 3 and 6 months, subjects were asked to return any remaining capsules, and the degree of apparent compliance was estimated from the number of delivered capsules and the number of returned capsules. Blood and urine samples were taken prior to supplementation and after 6 months of Zn supplementation. Blood samples were collected at 08.00 hours after an overnight fast, by venepuncture into EDTA, in heparinized, trace element-free heparinized and non-anticoagulant-containing tubes for Zn, Cu, Fe and vitamin E status assessment, *in vitro* Cu-induced LDL oxidizability and serum lipid profile determination.

Subjects

A total of ninety-six healthy subjects (forty-eight females and forty-eight males, aged 55–70 years) participated in the present study. Clinical examination was carried out by the medical staff at the Unit of Nutritional Exploration (UEN) of the Human Nutrition Research Center (CRNH) of the Auvergne, in France. All subjects were non-smokers and were judged to be healthy on the basis of a physical examination, medical history and a routine blood screening. At the time of recruitment and throughout the duration of the study, none of the enrolled subjects took vitamin or mineral supplements or medications which may have affected mineral metabolism. Dietary intakes of Zn, Cu, Fe and vitamin E were estimated using 4 d food-intake records. Participants were asked to maintain their habitual diet and exercise patterns for the duration of the study. The study was approved by the local human ethical committee (Comité Consultatif de Protection des Personnes en Recherche Biomedicale Auvergne, Clermont-Ferrand, France) under number AU 478. All participants were fully informed of the objectives of the study and gave their written consent. Three groups of thirty-two people (sixteen females and sixteen males in each group) were randomly assigned to the experimental design; group 1 received a placebo, group 2 received 15 mg Zn/d and group 3 received 30 mg Zn/d.

Dietary intake of micronutrients: Zn, Cu, Fe and vitamin E

At the beginning and at the end of each supplementation period, dietary intakes of micronutrients were estimated using 4 d food-intake records (including weekend). Each volunteer had received a notebook with one table per meal or snack to fill in. They had to note for each meal or snack, the names of foods and drinks, and the quantities ingested by an estimation using domestic measurements. The dietary data were checked with a dietitian, using a picture book for the estimations of quantities which has been validated (Le Moullec *et al.* 1996). Using the correspondence between the reference of the picture and the weight of food given in a table, all the dietary data were converted into grames of food or drink ingested. Then, nutrient intakes were estimated using the dietetics software 'GENI' (Gestion des Enquêtes Nutritionnelles Informatisée; Micro 6, Villers-lès-Nancy, France), whose food composition table is derived from the *Répertoire général des aliments* (Favier *et al.* 1995).

Status biomarkers of micronutrients: Zn, Cu, Fe and vitamin E

The trace element-free heparinized or non-anticoagulant-containing blood tubes were centrifuged (1000 ***g***, 10 min, 4°C) and serum, plasma and erythrocytes were collected and stored at −20°C until analysis. First morning urine samples were collected after a 12 h fasting period. Samples were then acidified with pure HCl (final acid concentration: 1 %) and stored at −20°C until analysis. For Zn analysis, serum and urine were diluted in 0·1 M-HCl (1:5, v/v) and erythrocytes were diluted in de-ionized water (1:100, v/v) and Zn concentration was determined on a flame atomic absorption spectrophotometer (560; Perkin-Elmer, Paris, France) at 214 nm (Arnaud *et al.* 1986). Urinary creatinine concentrations were measured by colorimetry (Hill *et al.* 2005) and the ratio of Zn to creatinine was

calculated. Serum Fe, ferritin and transferrin saturation and serum Cu levels and erythrocyte superoxide dismutase activity were measured as previously described (Andriollo-Sanchez *et al.* 2005). Plasma vitamin E level was determined by HPLC as previously described by Maiani *et al.* (1989).

Serum lipid profile

Serum samples were obtained from blood drawn in anticoagulant tubes for lipid, determination following centrifugation (1000 g, 10 min, 4°C), and stored at −80°C until analysis. Serum triacylglycerols, total cholesterol, LDL-cholesterol and HDL-cholesterol were determined by photometric analysis on a Hitachi 912 Clinical Chemistry Autoanalyzer, using commercially available kits purchased from Roche Diagnostics Ltd (Welwyn Garden City, UK).

LDL isolation and in vitro *oxidation*

LDL was isolated as follows (Rayssiguier *et al.* 1993): EDTA blood samples were centrifuged (1000 g, 10 min, 4°C) within 1 h of collection. Plasma was collected and mixed with a sucrose solution to a final concentration of 6 g/l (0·6 %), then stored at −80°C until analysis. Plasma was adjusted to a density of 1·019 g/ml with potassium bromide to extract VLDL and then to 1·063 g/ml to collect LDL. Ultra-centrifugation was performed twice at 15°C, at 645 000 g, for 2 h, in a Beckman Optima L-90 model ultracentrifuge (Beckman Instrument, Palo Alto, CA, USA) with a Beckman NVT 90 rotor. The yellow LDL fraction was removed and dialysed at 4°C in darkness for 24 h against 0·01 M-PBS (pH 7·4) containing 0·15 M-NaCl, which was made oxygen-free by vacuum degassing followed by purging with nitrogen.

In vitro oxidation experiments were performed on freshly prepared LDL as previously described (Feillet *et al.* 1998). Briefly, LDL was diluted with PBS to a final concentration of 50 μg protein/ml and oxidation was initiated by adding freshly prepared $CuCl_2$ (final concentration 5 μmol/l). Absorbance at 234 nm was continuously monitored for 8 h in the spectrophotometer (Uvikon 820; Kontron, St-Quentin-en-Yvelines, France) and oxidation was determined as the production of conjugated diene. Lag time, which represents resistance to oxidation, was determined as the intercept of the baseline and propagation phase of the absorbance curve.

Statistical analyses

Results are expressed as means and standard deviations. Statistical analyses were based on a two-way or a three-way repeated measure ANOVA with interactions on simple linear regression and on Spearman's correlation coefficients. The limit of statistical significance was set at $P<0·05$. Statistical analyses were performed using Statview software (SAS Institute Inc., Cary, NC, USA).

Results

Subject characteristics

As shown in Table 1, no significant differences were observed between the three groups according to sex and in terms of age, height, body weight and BMI. Of the ninety-six recruited subjects, ninety-five subjects completed the 6-month Zn supplementation study. Only one subject dropped out before the end of the trial (a woman in group 3). Analysis of the returned capsules revealed that compliance was greater than 98 %.

Dietary intakes of micronutrients: zinc, copper, iron and vitamin E

As shown in Table 2, daily dietary intake of Zn (about 13 mg in men and 10 mg in women), Cu (about 1·3 mg in men and 1·1 mg in women), Fe (about 14 mg in men and 10 mg in women) and vitamin E (about 11 mg in men and 9 mg in women), may be considered as adequate in this population compared to the French RDA for this age range. As already reported (Galan *et al.* 2005), the dietary intake of these micronutrients was significantly higher in men than in women. As expected, there were no significant differences in the dietary intake of these micronutrients with regards to the Zn administered doses or the period of treatment.

Status biomarkers of micronutrients: zinc, copper, iron and vitamin E

Serum Zn levels were similar in the three groups in both sexes before the Zn supplementation. As expected, serum Zn levels increased significantly ($P<0·001$) following Zn supplementation (Table 3). Erythrocyte Zn level was significantly higher ($P < 0·03$) in men than in women, and it remained unchanged after Zn supplementation. Urinary Zn excretion

Table 1. Characteristics of study population at the beginning of the study (T0)*
(Mean values and standard deviations)

	Placebo (group 1)				15 mg Zn/d (group 2)				30 mg Zn/d (group 3)				*P* value†		
	Men		Women		Men		Women		Men		Women				
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Effect of sex	Effect of doses	Interaction (sex × group)
Age (years)	62·9	3·4	61·4	4·6	59·9	4·0	61·3	4·5	61·3	4·6	60·9	4·4	NS	NS	NS
Body weight (kg)	77·8	8·5	65·5	8·2	77·2	8·8	65·6	8·1	75·7	9·3	67·0	6·7	<0·0001	NS	NS
Height (m)	1·72	0·07	1·59	0·06	1·73	0·06	1·61	0·06	1·71	0·06	1·59	0·06	<0·0001	NS	NS
BMI (kg/m^2)	26·4	2·3	25·9	2·8	25·9	2·7	25·5	2·3	26·1	2·2	26·4	2·1	NS	NS	NS

* *n* 16 men and 16 women (except group 3, where *n* 16 men and 15 women).
† A two-way repeated measure ANOVA with interactions was performed for these parameters. Statistical significance was set at $P<0·05$.

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Table 2. Dietary intakes of zinc, copper, iron and vitamin E at the beginning of the study (T0) and after 6-month zinc supplementation (T6)*
(Mean values and standard deviations)

	Placebo (group 1)				15 mg Zn/d (group 2)				30 mg Zn/d (group 3)				*P* value†			
	T0		T6		T0		T6		T0		T6					
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Effect of sex	Effect of doses	Effect of period	Interaction (sex × group × period)
Zn intake (mg/d)													<0·0001	NS	NS	NS
Men	13·2	2·5	13·3	2·5	12·4	3·4	15·0	4·6	13·2	3·5	11·7	2·8				
Women	10·2	2·4	9·7	3·4	11·7	8·3	9·7	2·3	9·5	2·9	9·5	2·8				
Cu intake (mg/d)													0·019	NS	NS	NS
Men	1·19	0·32	1·35	0·38	1·11	0·46	1·46	0·55	1·22	0·48	1·35	0·60				
Women	1·10	0·24	1·22	0·84	1·09	0·54	1·08	0·37	1·14	0·63	1·01	0·34				
Fe intake (mg/d)													<0·0001	NS	NS	NS
Men	12·9	3·0	13·4	2·9	14·8	3·1	15·5	4·0	13·6	4·0	15·7	6·7				
Women	9·8	2·2	10·4	3·6	11·1	4·3	9·6	2·4	10·8	2·2	9·1	2·0				
Vitamin E intake (mg/d)													0·03	NS	NS	NS
Men	10·5	3·9	9·3	3·5	12·1	6·9	11·0	5·7	11·3	7·8	10·0	4·1				
Women	10·0	4·5	9·2	3·6	10·2	5·3	9·3	4·4	8·1	3·8	7·9	3·1				

* *n* 16 men and 16 women (except group 3, where *n* 16 men and 15 women). For details of procedures, see p. 1135.
† A three-way repeated measure ANOVA with interactions was performed for these parameters. Statistical significance was set at $P<0·05$.

adjusted to urinary creatinine excretion also increased significantly ($P < 0·01$) over the 6 months of supplementation in the groups receiving 15 and 30 mg/d, with no significant change in the placebo group compared to their baseline values (Table 3).

Serum Fe level ranged from 16 to 22 μmol/l and serum transferrin saturation ranged from 32 to 43 %. There were no significant effects of sex or treatment on these two Fe biomarkers (Table 4). However, serum ferritin level, that ranged from 98 to 240 μg/l, was not affected by Zn supplementation, but was significantly higher ($P < 0·0001$) in men than in women. Serum Cu level was significantly higher ($P < 0·0005$) in women than in men whereas erythrocyte Zn,Cu-superoxide dismutase activity was significantly higher ($P < 0·02$) in men than in women. These two Cu status parameters remained unchanged after Zn supplementation. Finally, plasma vitamin E level and vitamin E:cholesterol were similar among the three groups at baseline and after 6 months of Zn supplementation. Moreover, there was no sex effect nor Zn dose effect on these two parameters.

Serum lipid profile and in vitro *LDL oxidation*

Serum lipid profile was similar among the three groups at baseline and after 6 months of Zn supplementation. When both sexes were compared, serum total cholesterol and HDL-cholesterol concentrations were higher in women than in men (Table 5).

Regarding the susceptibility of LDL to *in vitro* oxidation, there were no significant differences between the three groups at the beginning of the study (Table 6). After 6 months of Zn supplementation, there was no change in Cu-induced LDL oxidation compared to the baseline values, and no sex effect was observed. Moreover, no significant correlations were found ($P>0·05$) between serum Zn levels ($r = -0·062, -0·025, -0·028$), plasma vitamin E levels ($r = -0·085, -0·022, +0·121$) or plasma vitamin E: cholesterol ($r = -0·021, -0·009, +0·126$) and the susceptibility of LDL to *in vitro* oxidation, for basal conjugated diene, maximal conjugated diene and lag time, respectively.

Discussion

Among essential trace elements, Zn has been shown to possess antioxidant properties, acting to protect sulphydryl groups against oxidation and participating in the inhibition of free radical production in the Haber–Weiss cycle by competing with transition metals (Bray & Bettger, 1990; Coudray *et al.* 1993; Powell, 2000). Thus, it can be postulated that an optimal Zn status would be a protective factor against deleterious oxidative effects such as lipid peroxidation and LDL oxidation. Low dietary Zn intakes have been reported in late middle-aged and elderly subjects (Briefel *et al.* 2000) but little is known about the antioxidant effects of Zn supplementation in these populations. Given that some studies have observed enhanced susceptibility of lipoproteins to oxidation in elderly people compared to young subjects (Khalil *et al.* 1996; Napoli *et al.* 1997), we hypothesized that an improvement of Zn status would lead to a decreased susceptibility of LDL to oxidation. Therefore, we investigated the effect of long-term moderate Zn supplementation on the *in vitro* oxidation of LDL in women and men. Our results clearly showed that a 6-month Zn supplementation at a dose of 15 or 30 mg Zn/d, as gluconate, did not affect the $CuCl_2$-induced *in vitro* LDL oxidation. There was also no significant sex difference in LDL oxidizability measures.

The antioxidant and the pro-oxidant properties of Zn, Fe, Cu and vitamin E may influence the behaviour and the *in vivo* LDL composition and thus possibly the *in vitro* LDL

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Table 3. Zinc status biomarkers at the beginning of the study (T0) and after 6-month zinc supplementation (T6)*

(Mean values and standard deviations)

	Placebo (group 1)				15 mg Zn/d (group 2)				30 mg Zn/d (group 3)				*P* value†			
	T0		T6		T0		T6		T0		T6					
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Effect of sex	Effect of doses	Effect of period	Interaction (sex × group × period)
Serum Zn (μmol/l)													0·038	0·0035	<0·0001	NS
Men	13·3	2·0	14·2	1·7	12·4	1·4	15·0	2·2	13·1	1·4	17·2	3·5				
Women	13·1	1·5	13·0	1·6	12·6	1·4	14·6	2·9	13·1	1·8	15·1	2·3				
Erythrocyte Zn (μmol/l)													0·026	NS	NS	NS
Men	236	64	251	51	224	59	227	52	244	74	257	61				
Women	231	67	235	64	212	53	241	71	192	49	211	46				
Urine Zn:creatinine (μmol/mmol)													NS	NS	0·005	NS
Men	0·72	0·33	0·77	0·39	0·67	0·30	0·77	0·25	0·62	0·28	0·88	0·38				
Women	0·72	0·46	0·54	0·21	0·61	0·36	0·80	0·41	0·60	0·32	1·07	0·48				

* *n* 16 men and 16 women (except group 3, where *n* 16 men and 15 women). For details of procedures, see p. 1135.
† A three-way repeated measure ANOVA with interactions was performed for these parameters. Statistical significance was set at $P<0·05$.

oxidation. So, the evaluation of the dietary intake and the status of these micronutrients in studied subjects appear necessary. The daily dietary intake of these micronutrients was adequate in this population as compared to the current French RDA (9·5 mg/d for women and 11·5 for men for Zn, 9 mg/d for women and men for Fe, 1·5 mg/d for women and men for Cu and 12 mg/d for women and men for vitamin E) for this age range (Arnaud, 2001; Azaïs-Braesco *et al.* 2001; Coudray, 2001; Coudray & Hercberg, 2001). Moreover, the status biomarkers of these three micronutrients were also adequate compared to the values reported in the literature. These status biomarkers were not affected by Zn supplementation. However, serum Zn levels were significantly increased in women and men at both doses, indicating that this lack of effect on LDL oxidation was not owing to poor compliance. Other studies have also demonstrated no effect of Zn supplementation on LDL susceptibility to oxidation in both diabetic patients (Blostein-Fujii *et al.* 1997) and healthy subjects (Gatto & Samman, 1995). Conversely, it has been observed that Zn supplementation led to decreased thiobarbituric acid reactive substance (TBARS) levels in diabetic patients (Anderson *et al.* 2001; Roussel *et al.* 2003) and lowered some markers of oxidative stress, such as malondialdehyde, hydroxynonenal and 8-oxo-D-guanidine, in healthy subjects. Some nutritional antioxidant supplementation studies such as carotenoid supplementation (Hininger *et al.* 2001) or Cu supplementation (Turley *et al.* 2000) in healthy adult subjects failed to observed any significant modification in *in vitro* LDL oxidation measures, although improvement in particular stress oxidant status parameters was observed. However, in the present study, Zn supplementation did not lead to a significant decrease in plasma TBARS levels or in TBARS: cholesterol ratio (M Andriollo-Sanchez, I Hininger–Favier, N Meunier, JM O'Connor, G Maiani, C Coudray and AM Roussel, unpublished results). Many hypotheses may be put forward to explain the lack of a protective effect of Zn supplementation on LDL oxidation in the present study. Firstly, the increase in serum Zn following Zn supplementation was not strong enough to induce a decrease in LDL oxidation. In our subjects, serum Zn increased by 18 % at 15 mg Zn/d and by 24 % at 30 mg Zn/d after 6 months of Zn supplementation. In another study, Gatto & Samman (1995) gave 50 mg Zn as sulphate for 4 weeks and obtained an 18 % increase in serum Zn. They also failed to observe changes in the oxidizability of LDL in their ten young male volunteers (aged about 24 years). Thus, the antioxidant effect of Zn in relation to LDL oxidizability was not demonstrated in man at 50 mg Zn/d, and higher doses of Zn are unlikely to be used, given the possible adverse interactions with Cu and Fe metabolism. It is also important to note that the studied subjects presented with normal serum Zn values (>10·7 μmol/l; Gibson, 1990) before Zn supplementation; therefore, Zn supplementation does not appear to be beneficial in terms of further improving antioxidant status of these populations as shown by the non-significant modification of plasma TBARS level (M Andriollo-Sanchez, I Hininger–Favier, N Meunier, JM O'Connor, G Maiani, C Coudray and AM Roussel, unpublished results) and in *in vitro* LDL oxidizability reported in the present study. It is also possible that the *in vitro* oxidation induced by Cu could be particularly drastic and that LDL oxidation induced by other more physiological or mild inducers, such as

Table 4. Status biomarkers of iron, copper and vitamin E at the beginning of the study (T0) and after 6-month zinc supplementation (T6)*
(Mean values and standard deviations)

Parameter	Placebo (group 1)				15 mg Zn/d (group 2)				30 mg Zn/d (group 3)				P value†			
	T0		T6		T0		T6		T0		T6					
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Effect of sex	Effect of doses	Effect of period	Interaction (sex × group × period)
Serum Fe (μmol/l)													NS	0·023	NS	NS
Men	19·4	4·3	19·0	4·9	19·6	5·0	18·7	4·5	22·6	7·6	19·2	4·9				
Women	17·6	4·0	17·9	4·5	17·8	5·7	16·8	5·1	20·9	6·2	19·5	4·3				
Serum ferritin (μg/l)													<0·0001	NS	NS	NS
Men	176	139	193	148	145	121	161	113	202	128	240	157				
Women	98	85	105	115	107	65	115	64	110	68	138	86				
Serum transferrin saturation (%)													NS	0·016	NS	NS
Men	33·5	9·4	33·6	9·8	36·4	10·9	34·3	9·9	42·7	16·5	37·1	11·6				
Women	32·1	8·3	33·9	8·7	34·3	13·4	34·4	13·1	38·1	11·0	37·8	9·6				
Serum Cu (μmol/l)													0·0001	NS	<0·0001	NS
Men	15·2	2·2	19·5	4·6	17·8	4·8	20·8	3·1	16·0	3·2	18·9	3·8				
Women	18·8	5·7	23·6	5·2	18·3	5·2	21·7	4·1	18·5	4·1	22·2	4·2				
Erythrocyte Zn,Cu-superoxide dismutase (U/g Hb)													0·0145	NS	NS	NS
Men	997	264	975	290	993	267	971	270	1024	300	1004	283				
Women	843	179	829	160	923	234	881	219	999	189	970	189				
Serum vitamin E (μmol/l)													NS	NS	0·0035	NS
Men	27·8	5·0	26·6	4·9	27·6	4·8	24·8	5·4	29·1	5·8	24·1	3·8				
Women	29·0	4·8	25·5	5·5	30·0	5·9	28·8	7·6	27·3	5·5	27·1	4·2				
Vitamin E:cholesterol (μmol/mmol)													NS	NS	0·001	NS
Men	4·91	0·70	4·57	0·84	4·69	0·61	4·16	0·94	4·90	0·64	4·26	0·65				
Women	4·96	0·70	4·33	0·81	4·75	1·03	4·51	1·46	4·40	0·76	4·33	0·63				

* *n* 16 men and 16 women (except group 3, where *n* 16 men and 15 women). For details of procedures, see p. 1135.
† A three-way repeated measure ANOVA with interactions was performed for these parameters. Statistical significance was set at $P<0·05$.

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Table 5. Lipid profile at the beginning of the study (T0) and after 6-month zinc supplementation (T6)*
(Mean values and standard deviations)

	Placebo (group 1)				15 mg Zn/d (group 2)				30 mg Zn/d (group 3)				*P* value†			
	T0		T6		T0		T6		T0		T6					
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Effect of sex	Effect of doses	Effect of period	Interaction (sex × group × period)
Triacylglycerols (mmol/l)													NS	NS	NS	NS
Men	1·21	0·43	1·33	0·93	1·03	0·40	1·08	0·51	1·17	0·86	1·19	0·68				
Women	1·00	0·26	1·00	0·67	0·93	0·30	1·04	0·37	1·09	0·48	1·25	0·51				
Cholesterol (mmol/l)													0·014	NS	NS	NS
Men	5·70	0·87	5·87	0·81	5·90	0·95	6·04	0·95	5·96	1·08	5·70	0·78				
Women	5·91	1·12	5·98	1·18	6·38	0·94	6·50	1·12	6·28	1·31	6·32	1·09				
LDL-cholesterol (mmol/l)													NS	NS	NS	NS
Men	3·60	0·69	3·69	0·71	3·80	0·87	3·83	0·89	3·99	0·92	3·73	0·71				
Women	3·69	0·94	3·69	1·07	4·24	0·90	4·31	1·08	3·92	1·12	4·04	0·97				
HDL-cholesterol (mmol/l)													0·0007	NS	NS	NS
Men	1·56	0·47	1·58	0·42	1·66	0·43	1·72	0·59	1·43	0·25	1·43	0·28				
Women	1·78	0·29	1·84	0·26	1·70	0·45	1·72	0·47	1·86	0·43	1·72	0·39				
LDL:HDL													NS	NS	NS	NS
Men	2·48	0·77	2·51	0·82	2·43	0·80	2·48	1·03	2·86	0·81	2·72	0·78				
Women	2·11	0·58	2·04	0·65	2·73	1·11	2·76	1·20	2·34	0·84	2·47	0·77				

* *n* 16 men and 16 women (except group 3, where *n* 16 men and 15 women). For details of procedures, see pp. 1135–1136.
† A three-way repeated measure ANOVA with interactions was performed for these parameters. Statistical significance was set at $P<0·05$.

Table 6. LDL susceptibility to *in vitro* copper-induced oxidation at the beginning of the study (T0) and after 6-month zinc supplementation (T6)*
(Mean values and standard deviations)

	Placebo (group 1)				15 mg Zn/d (group 2)				30 mg Zn/d (group 3)				*P* value†			
	T0		T6		T0		T6		T0		T6					
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Effect of sex	Effect of doses	Effect of period	Interaction (sex × group × period)
Baseline diene (μmol/g protein)													NS	NS	NS	NS
Men	254	76	234	68	234	114	248	120	218	76	224	68				
Women	230	138	226	114	210	44	226	48	262	88	254	72				
Lag time (min)													NS	NS	NS	NS
Men	94·0	14·1	96·2	13·1	95·3	11·6	96·6	19·0	95·4	9·4	95·3	10·1				
Women	89·7	9·9	93·0	10·1	93·5	10·7	96·8	14·3	90·4	11·2	93·7	12·3				
Diene production (μmol/g protein)													NS	NS	NS	NS
Men	696	92	690	92	722	220	736	290	706	140	682	108				
Women	746	282	708	208	702	156	688	148	820	238	756	156				

* *n* 16 men and 16 women (except group 3, where *n* 16 men and 15 women). For details of procedures, see pp. 1135–1136.
† A three-way repeated measure ANOVA with interactions was performed for these parameters. Statistical significance was set at $P<0·05$.

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haem-iron (Grinshtein *et al.* 2003; Klouche *et al.* 2004) or monocytes–macrophages (Wilkins & Leake, 1994; Chisolm *et al.* 1999), may be more appropriate. Consequently, new approaches need to be developed to determine LDL oxidizability with mild inducers, and preferably in their biological medium without isolation and where LDL oxidizability can be measured without elimination of the many protective molecules that LDL may contain when they are in their biological medium.

The susceptibility of LDL to oxidation is influenced also by inherent LDL composition, which has been shown to determine the degree of resistance to oxidative stress. Indeed, it is well known that Zn status modulates lipid metabolism (Hiller *et al.* 1995; Schlegel-Zawadzka *et al.* 2004) and Zn supplementation may lead to modifications in the lipid composition of LDL, in particular fatty acids. Such modifications may, in turn, modify LDL oxidizability. In the present study, we observed no modification in serum cholesterol, expressed as total or lipoprotein-cholesterol, nor in serum triacylglycerol levels in Zn-supplemented groups; but fatty acid composition was not determined. It has been reported that moderate Zn supplementation did not modify lipid profiles in a late-middle-aged population (Bonham *et al.* 2003), which is in agreement with our results. However, other studies have shown significant modifications in lipid profiles under Zn supplementation (Hooper *et al.* 1980; Freeland-Graves *et al.* 1982; Chandra, 1984; Black *et al.* 1988) that might be explained by the higher doses of supplemental Zn given in these studies. LDL resistance to oxidation also depends on the presence of antioxidants together with substrates for oxidation in the lipoprotein. Plasma vitamin E, which plays an important role in the kinetics of lipoprotein oxidation, remained constant throughout the study, as previously reported (Gatto & Samman, 1995). This shows that Zn supplementation was not efficient in preserving plasma vitamin E and may partially explain why Zn supplementation was not efficient in attenuating *in vitro* LDL oxidation in the present study.

In conclusion, although Zn status significantly improved in the present study, our results showed that a 6-month Zn supplementation at moderate doses in healthy French subjects aged 55–70 years had no effect on *in vitro* Cu-induced LDL oxidation. This may be owing to adequate Zn status of the subjects at the beginning of the study and supplemental Zn that was thus not efficient in further improving antioxidant status of those subjects.

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