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Supplemented zinc does not alter mood in healthy older European adults – a randomised placebo-controlled trial: the Zenith study

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Abstract

Objective: Older people are vulnerable to zinc deficiency, which may impact upon their mood. This randomised, placebo-controlled, double-blind intervention study aimed to investigate the effect of oral zinc gluconate supplementation (15 mg/d; 30 mg/d; and placebo) on subjective mood (affect) in older Europeans.
Subjects: Healthy volunteers (*n* 387) aged 55–87 years were recruited.
Setting: Volunteers in Rome (Italy; *n* 108) and Grenoble (France; *n* 91) were aged 70–87 years and those in Coleraine (Northern Ireland; *n* 93) and Clermont-Ferrand (France; *n* 95) were aged 55–70 years.
Design: Mood was measured using the Positive and Negative Affect Scale on four occasions per day over 4 d at baseline, 3 and 6 months post-intervention.
Results: Mixed ANOVA indicated that neither positive nor negative affect altered in response to zinc (15 mg/d or 30 mg/d) compared to placebo in either the 55–70 years or the ≥70 years age group.
Conclusions: These results suggest that zinc does not benefit mood in healthy older people.

Keywords
Zinc
Intervention
Mood
Affect
PANAS
Elderly
Zenith

Psychological well-being is high on the list of public health policy priorities, with the emphasis on prevention[1]. Zinc is an essential trace element[2] that is present in large quantities in the brain and may be important for the maintenance of psychological well-being. Zinc-containing neurons are found in the forebrain interconnecting the cerebral cortex (the cognitive or 'thinking' part of the brain) and the limbic system (the affective or 'feeling' part of the brain)[3]. The synaptic vesicles of the hippocampus and amygdala, which are both limbic structures, contain particularly large quantities of zinc[4], implying a role for zinc in the control of emotion. Zinc-containing neurons are receptive to glutamine, an amino acid neurotransmitter that is excitatory and that exerts a neuromodulatory effect on post-synaptic glutamate receptors within the hypothalamic–pituitary–adrenal axis (HPA) system[4,5]. Rodent studies have indicated that this action occurs mainly through the hippocampus synapses, which are involved in the regulation of the HPA system[6–11]. Zinc may also be involved in the control of the serotonergic system[5,12,13]. Zinc has been shown to increase the density of 5-HT serotonin receptors in the hippocampus and frontal cortex in rats[12]. Post-mortem evidence suggests that zinc may have a role in the nutritional methylation processes[14] that are also believed to be important in the regulation of mood[15]. The human central nervous system appears to be adversely affected by zinc deficiency. Lower serum zinc is associated with depression[16–20] and a marker of treatment resistance in clinical depression[20,21]. Lower serum zinc is also associated with disordered behaviour[22–24]. Zinc supplementation has also been shown to ameliorate depression in clinical groups[25,26]. There is an apparent dearth of human studies of zinc and well-being in healthy (non-clinical) groups. Among the few studies of healthy individuals is a recent cross-sectional study of Korean women (*n* 570), which indicated that among other nutrients and trace elements, lower dietary zinc intake was associated with higher stress scores on the short version of the Psychological Well-being Index[27]. A recent double-blind, placebo-controlled trial of zinc (7 mg/d) in young women (*n* 30) found reduced scores on the anger/hostility and depression scales of the Profile of Mood States[28]. These studies imply a link between zinc and psychological well-being in health. There appear to be no existing trials of zinc and well-being or mood in men and/or older people.

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Europe and much of the industrialised Western world has a growing ageing population[1], hence, the need to maintain psychological well-being and quality of life in this vulnerable age group. The elderly are particularly at risk of zinc deficiency, the likelihood of which increases with age[29–33] as a result of a range of physiological, social, psychological and economic factors[34] that may be associated with the decreased consumption of zinc-rich foods[35–38]. Dysregulation of the HPA system is associated with the increased risk of CVD, diabetes, cancer, inflammatory conditions and neurodegenerative disorders[39]. HPA system activity has been shown previously to be related to positive affect (PA) in the elderly[40]. Maintaining a higher PA in old age, therefore, is potentially important for general health and well-being as well as disease prevention[41] in otherwise healthy older people. Assuming that zinc exerts a neuromodulatory effect through the HPA system, optimising zinc status in older people, therefore, is likely to have far-reaching benefits to psychological health. Yet, there do not appear to be any previous studies that have considered zinc and affect in healthy older individuals. Existing evidence for benefits of zinc to psychological well-being is mainly derived from *post hoc* investigation using psychiatric in-patients. Trials in healthy populations are necessary to further our understanding of how dietary constituents such as zinc impact upon health and well-being and to provide evidence to support or refute potential functional claims for zinc-rich food products. Trials in healthy individuals are especially important, given the widespread and increasing use of self-prescribed nutrient supplements by the older public[42]. The Zenith Study aimed to assess the effects of zinc supplementation in the normal healthy older population. It was necessary, therefore, to intervene with zinc at dosages that would be available for purchase over the counter without prescription. This randomised, placebo-controlled, double-blind intervention study reports the effect upon PA and negative affect (NA) of zinc (15 mg/d and 30 mg/d) supplements administered orally over 6 months compared to placebo in healthy 55–70-year-olds recruited to the Zenith Study in Coleraine and Clermont-Ferrand and ≥70-year-olds in Grenoble and Rome. This research meets an imperative for controlled supplementation trials investigating the effect of zinc on psychological well-being in the older population. It is hypothesised that zinc supplementation will alter subjective affective state compared to placebo.

Methods

The Zenith Study was a randomised, double-blind, placebo-controlled intervention trial of zinc in older adults, which was conducted in four European centres. Ethics approval for the study was obtained from recognised research centres in each country. Informed, written consent was obtained from each participant.

Sampling

Volunteers were outreached in different ways including through local television and radio broadcast, posters and through leaflets distributed in supermarkets, clubs that free-living elderly people frequently visit, health centres, and family doctors. Prospective volunteers then initiated contact with the research group who then arranged an appointment for the preliminary screening. The volunteers were screened first by clinical examination, anthropometry, smoking and alcohol consumption, cognitive impairment, depression and biochemistry profiles. Those selected for inclusion in the study were apparently healthy, not morbidly obese and with a BMI of between 20 kg/m^2 and 30 kg/m^2[43]. Only those who had no evidence of dementia, as indicated by a score >23 in the Mini Mental State Examination (MMSE) test[44], were included. Volunteers were screened for depression using the Geriatric Depression Scale (GDS)[45]. The GDS has been validated as a screening tool for depression against the DSM-IV criteria according to which only those scoring ≥6 can be considered not clinically depressed[46]. Those with a score of ≤5 were included. Participants were also excluded if they had a positive serology for HIV or hepatitis C, if they smoked more than ten cigarettes per day; consumed alcohol >30 g/d for men and >20 g/d for women; had unusual dietary habits (vegetarianism and veganism); used a mineral supplement in the 3 months preceding the study; used more than three drugs daily if aged 55–70 years; used more than four drugs daily if over 70 years; used drugs including antidepressants, laxatives and hormone replacement therapy; or had a pathological disease (cancer, diabetes, insufficient renal or hepatic performance, malabsorption and inflammatory chronic pathologies). Complete details about screening and recruitment processes have been described previously by Polito *et al.*[47].

Serum zinc

Unfortunately, there is no single accurate measure of zinc status[48,49]. For the purpose of the present study, serum zinc was taken as a putative indicator of current zinc status[50]. Fasting blood samples were obtained for the zinc assay in the morning. Blood samples for the zinc assay were collected via venepuncture by qualified the phlebotomists, using trace element-free Vacutainer® tubes (Becton, Dickinson and company Ltd, Oxford, UK). The blood sample was taken in the morning after a 12 h fast by the participants. Immediately after drawing, the blood samples were kept on ice, and then centrifuged at 1000***g*** for 15 min at 4°C. Serum zinc was immediately isolated, aliquoted and stored at −80°C. Serum zinc concentrations at baseline were determined at INRA Clermont-Ferrand, France, by flame atomic absorption spectrometry using a Perkin-Elmer 560 instrument (Perkin Elmer, Cambridge, UK)[50]. Internal quality control was checked using Seronorm® trace element serum (Sero®, Billingstad, Norway).

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Dietary zinc

Dietary zinc intake was assessed at baseline by means of a semi-structured standardised 4 d food diary over two weekdays and two weekend days consecutively, which included estimates of portion size, the brand name of the product and/or the recipe if cooked from fresh. The information in the food diaries was analysed using the NetWISP version 3·0 (Tinuviel Software Anglesey, UK) database. A full account of the dietary assessment procedure has been provided previously by Polito *et al.*[51].

Positive and Negative Affect Scale

The Positive and Negative Affect Schedule (PANAS) is a well-validated twenty-item self-reported psychometric measure of subjective mood developed by Watson *et al.*[52]. Watson and Tellegen[53] proposed that there are two distinct dimensions of mood state, PA and NA, which together account for over two-thirds of the variance in mood. PA is associated with feelings of alertness, enthusiasm and happiness and NA with displeasure and dissatisfaction[54]. PANAS scores have been shown to correlate with salivary cortisol levels[40] and whole blood serotonin levels in healthy individuals[55,56]. The scales have been found to have high internal consistency, with Cronbach's α ranging from 0·84 to 0·87 for the NA scale and 0·84 to 0·90 for the PA scale[57]. Repeated measures of affect were taken during the week before the centre visit, including one further measure taken on the morning of testing. The participants were asked to complete four self-reported PANAS questionnaires daily (on rising, after lunch, after dinner, and before going to bed) for four consecutive days at baseline, 3 and 6 months. The scale took approximately 2 min to complete. The items were: interested; distressed; excited; upset; strong; guilty; scared; hostile; enthusiastic; proud; irritable; alert; ashamed; inspired; nervous; determined; attentive; jittery; active; and afraid. Responses to the PANAS were recorded on a 5-point Likert scale ranging from 'not at all' = 1 to 'extremely' = 5. A score for each scale was obtained by summing item scores.

Intervention

Eligible volunteers were randomly assigned to three groups to receive either 15 mg/d or 30 mg/d zinc gluconate or placebo orally. Zinc capsules were issued in dated pillboxes at baseline and at 3 and 6 months into the supplementation phase. The participants were instructed to swallow the capsules with breakfast. The degree of compliance was estimated by collecting the number of returned capsules at 3 and 6 months. Compliance was tested and was found to be >98 % for all groups.

Data analysis

The mood data were analysed using a mixed ANOVA. Separate analyses were computed for the 55–70-year-olds (n 188; from Coleraine and Clermont-Ferrand) and for the ≥70-year-olds (n 199; from Grenoble and Rome). There were three between-group factors (sex, centre and treatment) and one repeated measure (baseline, 3 and 6 months post-intervention) included in each ANOVA. Mean PA and NA were treated as separate dependent variables, which also required separate ANOVAs. In the absence of relevant previous studies in this area, a conservative value of $f^2 = 0{\cdot}025$ for effect sizes was adopted. For the mood scale it was assumed that the correlations among each of the repeated measures were 0·42 (taken from the PANAS manual). Power calculations were performed using GPOWER general power analysis computer program (G*Power, Bonn, Germany)[58]. With $\alpha = 0{\cdot}05$ and assuming 180 participants within each age group (55–70 and ≥70 years), the power values for the between-groups and within-groups and interaction effects were 0·36, 0·99 and 0·81, respectively. As the interaction effects (particularly the treatment–time interaction) were of prime concern in the present study, the sample sizes for both the younger (n 188) and the older (n 199) age groups were regarded as satisfactory. Significance was set at $P < 0{\cdot}05$. The Statistical Package for Social Sciences statistical software package version 11·5 (SPSS UK Ltd., Feltham, UK) was used for statistical analysis.

Results

Approximately 10–15 % of those who initiated contact were included in the study. Equal proportions of healthy male and female older volunteers were recruited from four European centres. Coleraine, Northern Ireland (n 93) and Clermont-Ferrand, France (n 95) recruited participants aged 55–70 years, while Rome, Italy (n 108) and Grenoble, France (n 91) recruited participants who were >70 years of age. In total, 387 participants successfully completed the trial (Table 1). A full description of the sample characteristics has been provided elsewhere by Simpson *et al.*[59].

Baseline measures

Neither sex nor age differed between the treatment groups. The younger (55–70 years) age group comprised a higher proportion (84 %) of professional occupations than the older (≥70 years) age group (68 %). A higher proportion of those in the younger age group (96 %) than the older age group (35 %) were educated to the tertiary level. Neither social class nor educational level differed between the treatment groups. There were no apparent differences by age group or treatment group in dietary zinc intake at baseline. There were no differences in MMSE or (GDS) scores by age group or treatment condition[60].

Serum zinc

Mean serum zinc was within the normal range (11–18 μmol/l)[61] for the placebo (mean = 13·20 (SD 1·69) μmol/l), 15 mg (mean = 13·28 (SD 1·84) μmol/l) and 30 mg (mean = 13·13 (SD 1·63) μmol/l) supplemented

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Table 1 Sociodemographic variables for each region, given as percentages

	Northern Ireland (55–70 years)		Clermont-Ferrand (55–70 years)		Rome (70–85 years)		Grenoble (70–87 years)	
Variables	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	62·4	4·5	61·3	4·3	74·5	4	74·2	3·5
	%		%		%		%	
Sex								
Male	48		51		52		52	
Female	52		49		48		48	
Education								
Primary	99		100		99		100	
Secondary	91		75		76		66	
Tertiary	41		35		20		15	
No education	1		–		1		–	
Social class								
Professional	54		30		15		41	
Skilled	33		60		58		53	
Unskilled	8		10		27		6	
Unclassified	4		–		–		–	

Total participants = 387: Northern Ireland = 93; Clermont-Ferrand = 95; Rome = 108; and Grenoble = 91.

Table 2 PANAS scores for each treatment group (55–70-year-olds/Coleraine and Clermont-Ferrand) over time (*n* 188)

	Positive affect						Negative affect					
	Placebo		Zn (15 mg)		Zn (30 mg)		Placebo		Zn (15 mg)		Zn (30 mg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	26·14	4·83	28·05	5·49	28·40	5·70	11·66	2·63	11·72	2·11	11·44	2·00
3 months	27·05	4·21	28·59	5·85	29·47	5·76	11·92	2·70	12·24	3·67	11·46	1·99
6 months	26·86	5·20	28·70	6·09	29·06	5·54	11·84	2·89	12·22	3·30	11·22	1·95

PANAS, Positive and Negative Affect Score.

groups at baseline and remained so throughout the intervention period. Serum zinc increased over time ($F(4197) = 11·021$, $P = 0·000$) in both the 15 mg (mean = 13·99 (SD 2·47) μmol/l; $P = 0·018$) and 30 mg (mean = 15·03 (SD 3·17) μmol/l; $P = 0·000$) supplemented groups compared to placebo (mean = 13·05 (SD 1·66) μmol/l) suggesting compliance with the intervention. Serum zinc concentrations in response to zinc (30 mg) were higher among those recruited in Rome (mean = 16·26 (SD 3·41) μmol/l) than Grenoble (mean = 13·64 (SD 2·21) μmol/l) post-intervention ($F(4197) = 3·526$, $P = 0·008$).

Affect (the Positive and Negative Affect Scale) and zinc

There were no treatment (15 mg/d zinc; 30 mg/d zinc; or placebo) × time (baseline; 3 months; or 6 months post-intervention) or interaction effects, indicating that the treatment had no differential effects on affect (PANAS) over time. For NA among the 55–70-year-olds, the interaction effect was $F(4340) = 1·56$, $P = 0·185$ (Table 1) and for the ≥70-year-olds, $F(4372) = 0·466$, $P = 0·761$ (Table 2). Interaction effects for PA among the 55–70-year-olds were $F(4340) = 0·37$, $P = 0·833$ (Table 2) and among the ≥70-year-olds were $F(4372) = 1·32$, $P = 0·261$ (Table 3). None of the triple interaction effects involving treatment and time were significant.

Centre and affect (the Positive and Negative Affect Scale)

There was a significant treatment × centre interaction effect for NA among the ≥70-year age group ($F(1186) = 3·73$, $P = 0·025$). For the placebo, 15 mg/d and 30 mg/d treatment groups, the means were 11·45, 13·43 and 11·94, respectively, for Rome and 13·06, 12·83 and 14·38, respectively, for Grenoble. For Grenoble, the trend for NA was U-shaped showing a decrease at 3 months into the intervention and returning to baseline at 6 months. For Rome, the trend for NA was an inverted U-shape such that NA was increased at 3 months into the intervention and returning to baseline at 6 months. There were no significant interactions between treatment and centre for PA in either the 55–70-year or ≥70-year age group.

Gender and affect (the Positive and Negative Affect Scale)

With the exception of a significant gender × centre interaction effect for NA among the 55–70-year-olds ($F(1170) = 4·71$, $P = 0·031$), there were no significant main effects or interaction effects for gender. The significant gender × centre interaction effect was such that male volunteers reported higher NA than female volunteers in the Coleraine sample (mean = 12·00 and 11·39,

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Table 3 PANAS scores for each treatment group (≥70-year-olds/Rome and Grenoble) over time (*n* 199)

	Positive affect						Negative affect					
	Placebo		Zn (15 mg)		Zn (30 mg)		Placebo		Zn (15 mg)		Zn (30 mg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Baseline	22·54	8·23	22·74	6·78	23·25	7·86	11·96	2·74	12·96	3·72	13·05	4·0
3 months	24·59	8·65	23·78	5·96	23·59	7·62	12·25	3·21	13·22	3·72	13·41	4·42
6 months	24·36	9·02	23·20	6·50	23·59	7·68	12·29	3·02	13·27	4·90	12·81	4·26

PANAS, Positive and Negative Affect Score.

respectively), whereas the reverse was true in the Clermont-Ferrand sample (mean = 11·48 and 12·14).

Discussion

Studies of clinically depressed[18–24] and healthy[27,28] groups have suggested a link between zinc status and psychological well-being. Given evidence to suggest that for a multiplicity of reasons, ageing can be associated with zinc depletion[31], it was hypothesised that supplemented zinc would enhance mood in older people. It was, therefore, surprising to find no alteration in PA or NA in response to zinc (15 mg/d or 30 mg/d) compared to placebo in either the younger (55–70 years) or the older (≥70 years) age group, who had lower PA than the younger age group at baseline[61]. Both NA and PA were unaffected by zinc supplementation in either age group. In the older age group (≥70 years), NA increased among those in Rome and decreased among those in Grenoble 3 months into the intervention and returned to baseline in both groups at 6 months. This trend is difficult to explain. Although serum zinc was higher among those in Rome than Grenoble at 3 months, serum zinc is not an accurate indicator of zinc status and could have been altered in response to a range of factors unrelated to affect[49]. Increased serum zinc concentrations post-treatment, however, suggest good compliance with the intervention in both groups. The PANAS is a well-validated subjective (self-reported) psychometric measure of affect[52], which has been widely used and previously used in other studies involving elderly adults[62]. The PANAS has also been shown to correlate with the biochemical markers of affect[40,55,56]. This correlation indicates that although the scales measure subjective affect, the data generated can be considered sensitive to corresponding changes in neurochemical activity. The PANAS can, therefore, be assumed to have good concurrent validity for the measurement of affect. Although the PA data collected for the present study agree with those of the established norms, the NA scores appear to be less than those suggested by established norms for older people[52]. The relatively low NA reported by our sample at baseline may explain the lack of change in NA following zinc supplementation. The apparent lack of change in affect in response to zinc could also be because the participants were zinc replete[50]. Screening procedures were stringent and care was taken to recruit only physically and psychologically healthy older people to the Zenith Study. Health and well-being tend to be confounded with social factors. It is possible that in selecting only healthy individuals, those from socio-economically deprived segments of society have been excluded. That our sample was biased towards the higher social classes and those who had spent a relatively longer period in education (Table 1) may also explain the apparently good psychological well-being among our sample and lack of response to the intervention. The participants, by virtue of having volunteered, may not be entirely representative of the general population, potentially limiting the degree to which the findings can be extrapolated to the general older population. Those who have spent longer periods of time in education and those in more privileged social classes tend to have lower mortality and morbidity[1] and may, therefore, also have a greater chance of being recruited to such studies.

Conclusion

The present study seems to be the first randomised, double-blind, placebo-controlled intervention trial investigating the impact of supplemented zinc upon affect in healthy older adults. Intervention outcomes were assessed using the PANAS, which is a well-validated subjective (self-reported) psychometric measure of affect. The study was also strong in that it used a relatively large sample comprising late middle-aged and elderly individuals from both northern and southern European populations with adequate power with which to uphold the null hypothesis and within which sex and cultural effects were controlled. These data suggest that zinc supplementation at considered 15 mg/d or 30 mg/d does not alter mood in healthy elderly European adults.

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B.J.S.-K. contributed to the design of the study and drafted the paper; G.R. undertook the statistical analysis; E.E.A.S. undertook data collection and database development; J.M.O. coordinated research at University of Ulster; C.Mc.C. contributed to the mood protocol; A.P. supervised the data collection at INRAN; M.A.-S. supervised the data collection at Grenoble; C.C. supervised the data collection at INRA and coordinated Zenith; and J.J.S. contributed to the nutritional protocol and advised on the zinc intervention.

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