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Effects of zinc supplementation on cognitive function in healthy middle-aged and older adults: the ZENITH study

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A randomised double-blind placebo-controlled design was employed to investigate the effects of Zn supplementation on cognitive function in 387 healthy adults aged 55–87 years. Several measures of visual memory, working memory, attention and reaction time were obtained using the Cambridge Automated Neuropsychological Test Battery at baseline and then after 3 and 6 months of 0 (placebo), 15 or 30 mg Zn/d. Younger adults (<70 years) performed significantly better on all tests than older adults (>70 years), and performance improved with practice on some measures. For two out of eight dependent variables, there were significant interactions indicating a beneficial effect (at 3 months only) of both 15 and 30 mg/d on one measure of spatial working memory and a detrimental effect of 15 mg/d on one measure of attention. Further work is required to establish whether these findings generalise to older adults in poorer mental and physical health and with less adequate Zn intake and status than the present sample.

Zinc: Cognition: Ageing: Cambridge Automated Neuropsychological Test Battery

Dietary intake affects brain function (Fernstrom, 2000) but there remain gaps in our knowledge of the behavioural effects of many nutrients, particularly in older adults (Mocchegiani *et al.* 2005). There is considerable interest in the potential cognitive benefits from nutritional supplements (see McDaniel *et al.* 2002), with some encouraging results, for example, with B vitamins (Calvaresi & Bryan, 2001), vitamins C and E (Masaki *et al.* 2000) and folate (Joyal *et al.* 1993). One micronutrient that has received less attention is Zn, a non-toxic trace element essential for many biochemical activities and physiological functions (for reviews, see Hambidge, 2000; Salgueiro *et al.* 2000). As an antioxidant, Zn is important to the immune, reproductive and central nervous systems and is present in many areas of the brain, particularly in the hippocampus and amygdala (Takeda, 2000), influencing brain structure and function (Black, 1998; Sandstead, 2000, 2003). Zn can only be obtained through the diet (mostly from meat and fish), the results of Zn deficiency ranging from slower wound healing to delayed physical and cognitive development (Hambidge, 2000).

Inadequate Zn nutriture has been identified in both developing and developed countries, particularly in infants, pregnant and lactating women and older adults (Briefel *et al.* 2000; Penland, 2000; Sandstead, 2000). Zn supplementation studies in infants and pregnant women have demonstrated benefits in terms of the physical health, growth rate and cognitive development of the infants and babies born to the mothers taking supplements (for reviews, see Black, 1998; Bhatnagar & Taneja, 2001; Salgueiro *et al.* 2002). There have been fewer studies of Zn supplementation in children and adults and although improvements in cognitive function have been observed, methodological issues suggest that further data are required (Penland, 2000).

As a nutritionally vulnerable group – because of physiological, social, psychological and economic factors – older adults are especially at risk of Zn deficiency (Blumberg, 1997; McClain *et al.* 2002). Briefel *et al.* (2000) reported that only 44 % of adults over 70 years had an adequate intake of Zn, and Prasad *et al.* (1993) concluded from their study of independent-living older individuals that Zn

Abbreviations: CANTAB, Cambridge Automated Neuropsychological Test Battery; MMSE, mini-mental state examination; MTS, matching to sample visual search; PRM, pattern recognition memory; SWM, spatial working memory.

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deficiency posed a 'significant clinical problem'. In view of evidence from animal studies of links between reduced Zn in the hippocampus and age-related decline in spatial memory (for summaries, see Takeda, 2000; Mocchegiani *et al.* 2005), it seems surprising that there have been only two substantial studies of the effects of Zn on cognitive function in older adults. Ortega *et al.* (1997) examined 260 Spaniards aged 65–90 years and found that better cognitive scores were associated with greater dietary intake of a number of nutrients including Zn, suggesting that 'zinc may influence cognitive function'. However, the authors acknowledged that their design was unable to establish cause and effect or to exclude possible confounding factors. Yaffe *et al.* (2004) administered cognitive tests to 2166 adults aged 61–87 years, half of whom had been receiving Zn supplements (80 mg/d) for several years. There were no significant differences between groups but the authors noted several limitations to their study, including the lack of cognitive measures before supplementation, and the loss of 40 % of participants.

The present study aimed to assess the Zn status of healthy late middle-aged and older Europeans and to investigate the effects of Zn supplementation on their cognitive functioning. Note that even if the sample were not Zn deficient, there may be advantages in consuming some nutrients at levels beyond average requirements (Kiely *et al.* 2001). Moreover, we need to establish that supra-nutritional doses of Zn have no adverse effects. This is vital because of the growing use of dietary supplements, including amongst older individuals (Canter & Ernst, 2004), and the increasing availability of foods specifically fortified with Zn (for example, 'Minute Maid' 100 % pure squeezed smooth orange juice and raspberry with Zn; http://www.minutemaid.co.uk/MM-Raspberry.asp).

The design was a randomised, double-blind, placebo-controlled intervention trial in men and women assigned to one of three treatments: placebo, 15 mg Zn/d or 30 mg Zn/d for 6 months. Clinical and psychological examinations and blood and urine samples were taken before intervention, and after 3 and 6 months of Zn supplementation. Cognitive function was assessed by the Cambridge Automated Neuropsychological Test Battery (CANTAB; Morris *et al.* 1986; Sahakian & Owen, 1992).

Method

Participants

Participants were recruited through posters, leaflets, local television and radio, and community groups and organisations serving older individuals living independently. Two centres (Coleraine, UK and Clermont-Ferrand, France) recruited younger participants (55–70 years); two centres (Rome, Italy and Grenoble, France) recruited older participants (70–87 years). Volunteers (292 in the younger group; 550 in the older group) were invited to attend a preliminary session that included a full medical history and examination, anthropometric measurements, assessment of dietary habits, tobacco and alcohol consumption, and screening for cognitive impairment and depression (mini-mental state examination (MMSE; Folstein *et al.* 1975) and geriatric depression scale (Yesavage *et al.* 1983), respectively). Fully informed written consent was required before taking part in the study. Ethical approval was obtained from the appropriate ethics committee in each of the three countries involved in the study. All participants received their travel expenses and (with the exception of the Italian sample) a small honorarium for taking part in the study.

Participants were excluded from further involvement in the study according to the following criteria: (1) tobacco consumption of more than 10 g/d; (2) alcohol consumption of more than 30 (men) or 20 (women) g/d; (3) unconventional dietary habits (for example, vegetarians, vegans); (4) use of a mineral supplement during the preceding 3 months; (5) use of more than three (55–70 years) or four (70–87 years) prescription drugs per d; (6) use of antidepressants, laxatives, or hormone replacement therapy; (7) pathological diseases, including cancer and diabetes. Inclusion criteria were: (1) men and women aged between 55 and 87 years; (2) BMI between 20 and 30 kg/m^2; (3) good health; (4) MMSE score greater than 23; (5) geriatric depression scale score less than 6. For participants satisfying these criteria, a biochemistry profile was performed, which included a full blood profile and tests of kidney and liver function. On the basis of these data, participants were excluded if there was insufficient renal and hepatic performance, malabsorption or inflammatory chronic pathologies, and were included if there was negative serology for the HIV and hepatitis C viruses.

Of the 842 volunteers who were screened, 49 % were not selected for further participation. Pathological conditions and the use of medications represented the main reasons why participants were excluded, especially for the older adults (for full details, see Polito *et al.* 2005). This resulted in 433 participants (201 younger; 232 older) admitted to the supplementation phase. During the 6-month study, forty-three participants dropped out (thirteen younger; thirty older). Compliance was less than 80 % for three older participants and so they were also excluded. Thus, a total of 387 participants (188 younger; 199 older), with approximately equal numbers of males and females (196 and 191, respectively), successfully completed the full Zn supplementation study.

The majority of the 387 participants were retired from full-time work (68·1 % of the younger group; 95·0 % of the older group). Participants' education was categorised into four levels: no formal education; primary education; secondary education; tertiary education. The percentages of younger participants at each level of education were 0·0, 16·0, 46·3 and 37·8, respectively. The corresponding percentages of older participants were 0·5, 28·1, 53·3 and 18·1. As expected, younger participants received significantly more formal education than older participants (χ^2 21·89; df 3; $P<0·001$).

Participants were assigned to one of three levels of Zn supplementation (placebo or 0 mg/d, 15 mg/d and 30 mg/d) according to the same standardised random order in each centre. The larger dose was determined on the basis of an average adult intake of about 10 mg Zn/d and a tolerable upper intake level for adults of 40 mg Zn/d (Food and Nutrition Board & Institute of Medicine, 2000). Table 1 shows the distribution of participants as a function of age group, treatment and sex and provides background measures collected at baseline (i.e. before supplementation) for each age group and (subsequent) treatment. Participants' daily intake of Zn was estimated from their detailed records of food and drink consumed over a 4 d period (Andriollo-Sanchez *et al.* 2005). (Data were unavailable for five participants.) Serum,

Table 1. Numbers of younger and older participants assigned to each of three Zn treatments (0, 15 and 30 mg/d), with age, screening test scores and Zn measures at baseline

(Mean values and standard deviations)

	Younger						Older					
	0 mg/d		15 mg/d		30 mg/d		0 mg/d		15 mg/d		30 mg/d	
Measure	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
n	63		60		65		67		66		66	
Males (*n*)	32		30		31		35		34		34	
Females (*n*)	31		30		34		32		32		32	
Age (years)	62·3	4·09	61·4	4·55	61·7	4·57	74·2	3·58	74·3	3·91	74·6	3·89
MMSE	29·0	1·18	28·7	1·23	28·6	1·30	28·6	1·66	28·2	1·84	28·7	1·50
GDS	1·44	1·46	2·03	1·46	2·11	2·08	1·85	1·52	1·88	1·49	2·12	1·43
Zn intake (mg/d)	10·56	3·19	10·73	5·43	10·35	3·57	12·09	6·19	10·17	3·50	11·69	5·28
Serum Zn (μmol/l)	13·20	1·65	12·74	1·48	13·01	1·34	13·21	1·69	13·29	1·84	13·14	1·64
Erythrocyte Zn (μmol/l)	232·4	54·3	216·9	54·4	219·4	58·0	196·1	56·0	200·1	54·4	208·7	59·4
Urinary Zn (μmol/mmol creatinine)	0·72	0·37	0·63	0·28	0·61	0·25	1·13	1·12	1·12	0·72	0·86	0·38

MMSE, mini-mental state examination (Folstein *et al.* 1975); GDS, geriatric depression scale (Yesavage *et al.* 1983).
For details of subjects and procedures, see p. 753.

erythrocyte and urinary Zn levels were determined by flame atomic absorption spectrometry using standard methodology (Arnaud *et al.* 1986). (Data were unavailable for two, twelve and five participants, respectively.) Erythrocyte Zn levels are representative of long-term Zn status (Vitoux *et al.* 1999).

Cognitive measures (Cambridge Automated Neuropsychological Test Battery)

CANTAB was selected to provide measures of cognitive function for a number of reasons. It is sensitive to cognitive changes due to a wide range of brain disorders and medications (Fray & Robbins, 1996; Fray *et al.* 1996). The tests have brain-to-behaviour reliability (Luciana & Nelson, 2002). Parallel versions are available for repeated measures, with high test–retest reliability (Semple & Link, 1991; Louis *et al.* 1999). Construct validity has been obtained from studies of neurological and psychiatric patients with disorders affecting specific brain regions (Elliott & Sahakian, 1995; Owen *et al.* 1996). CANTAB has been used extensively in clinical trials (Louis *et al.* 1999) and in research with older adults (Robbins *et al.* 1994). The tests are graded to allow for a wide range of ability whilst avoiding floor and ceiling effects. CANTAB assesses cognition using a computer to ensure standardised presentation and feedback with non-verbal stimuli and touch-screen methodology, rendering it suitable for research across different European centres. Finally, both speed and accuracy are recorded (on the importance of this with regard to investigations of nutritional stressors, see Penland, 2000).

Visual memory was tested by pattern recognition memory (PRM; requiring temporal lobe–hippocampal–amygdala activation), working memory was tested by spatial span and spatial working memory (SWM; requiring both temporal and frontal lobe activation), and attention was tested by reaction time and matching to sample visual search (MTS; activating several brain regions including fronto-striatal circuitry) (for a summary of supportive evidence, see Robbins *et al.* 1997).

Procedure

Zn supplementation (0, 15 or 30 mg/d) was administered as zinc gluconate, participants taking two tablets at the same time each day (usually after breakfast) for 6 months. (Tablets were identified by a code so that neither the experimenter nor the participant knew the dose. The code was not broken until the study had been completed and all the data had been entered into computer files ready for analysis.) Cognitive function was assessed in the laboratory at baseline (before supplementation) and after 3 and 6 months of supplementation, using different (parallel) versions of the CANTAB tests on each occasion. Participants fasted overnight for 12 h before blood and urine samples were taken on each of these occasions to determine Zn levels.

The CANTAB tests were administered after participants had eaten breakfast in the laboratory and took approximately 35–40 min. Experimenters from each of the four testing centres had previously attended a 2 d training course at Cambridge Cognition Limited (Cambridge, UK), to ensure that the tests were administered in exactly the same way. In addition, standardised verbal instructions were taken verbatim from the CANTAB test manual, which was available in English, French and Italian.

The procedure was identical on each of the three testing occasions. Stimulus presentation was computer-controlled and responses were obtained using a touch-sensitive screen and also a large press-pad for some of the tests, placed 0·15 m from the screen. Participants were seated approximately 0·5 m from the screen. They were first introduced to the equipment and familiarised with the response methodology by completing a motor screening test: participants responded to a series of crosses on the screen by touching each one with the index finger of their preferred hand as soon as a cross appeared. If their response was accurate, auditory feedback was presented, the cross disappeared and the next one was presented after a short delay. Following successful completion of this task (ten crosses), the main cognitive tests were presented, always in the following order.

Pattern recognition memory. In the study phase, participants were shown a series of twelve simple abstract coloured

patterns that could not easily be assigned verbal labels. Each pattern was presented for 3 s and appeared inside a white box in the centre of the screen. In the test phase, twelve pairs of target-distractor patterns appeared serially, with one item to the left and the other to the right of the screen centre, and for each pair participants were required to touch the pattern seen during the study phase. Visual feedback on accuracy was provided (green tick or red cross for correct or incorrect responses). Distractor patterns differed from target patterns in form but not in colour. Targets in the test phase were presented in the reverse order to the original order at study. This entire procedure was then repeated with a new set of twelve patterns followed by twelve pairs of patterns for recognition. Both response accuracy (the total number of correctly recognised patterns out of twenty-four, expressed as a percentage) and mean latency in ms for correct trials were measured.

Spatial span. In this task, based on the Corsi Block Tapping task (Milner, 1971), a set of nine white boxes was shown on the screen. Some of these boxes then changed colour for 3 s one by one in a random sequence. A tone then indicated to participants that they should touch each of the highlighted boxes in the same order as they were originally coloured by the computer. The task began with a sequence of two boxes and then increased in length by one box each trial if the participant correctly recalled the sequence. After an incorrect attempt, another sequence of the same length was presented. This continued until the participant had failed three consecutive trials at any one length. Thus three attempts were allowed at each level up to a maximum of nine boxes. The dependent measure was the highest level at which the participant correctly recalled at least one sequence of boxes.

Spatial working memory. Participants were required to search through a set of red boxes on the screen in order to collect blue tokens hidden inside the boxes and use them to fill an empty column on the right-hand side of the screen. Touching a box revealed whether or not it contained a blue token. Participants were instructed that only one token would be hidden at a time and that their task was to search until it was found, at which point the next token would be hidden. It was repeatedly emphasised that once a blue token had been found, that particular box would not be used again to hide a token on that trial. Every box was used once to hide a token so the total number of blue tokens to be found on each trial was equal to the total number of red boxes displayed on the screen. There were four practice trials with three boxes and then twelve test trials with four, six and eight boxes (four trials at each level). The colour and position of the boxes used were changed from trial to trial to discourage the use of stereotyped search strategies.

A 'between-search error' was recorded when a participant returned to open a box in which a blue token had already been found in a previous search, whereas a 'within-search error' was recorded when a participant returned to a box already opened and shown to be empty in the same search sequence. The three levels of difficulty (four, six and eight boxes) were combined to produce the total number of errors (between + within) as the first dependent measure. Owen *et al.* (1990) identified a second dependent measure ('strategy') reflecting the extent to which an efficient search strategy was adopted. This involves a repetitive searching pattern in which participants always begin with a particular box, search the boxes in a fixed order, and then return to start each new sequence with the same box when a token has been found. A strategy score was determined on the basis of trials with six and eight boxes, with higher scores reflecting poorer use of the strategy (less efficient search) than lower scores.

Reaction time. There were two conditions in this task, namely, simple reaction time and five-choice reaction time, and they were always performed in that order. In the simple reaction time task, participants were required to hold down the large press-pad in front of them until a yellow dot appeared inside a circle in the centre of the computer screen. At this point, participants had to release the press-pad and touch the yellow dot as quickly as possible. The next dot appeared only after participants had returned to hold down the press-pad again. In the five-choice reaction time task, the yellow dot could appear in any one of five circles arranged around the centre of the screen. Both tasks were divided into practice and test phases, and participants were required to obtain nine out of ten trials correct in the practice phase before progressing to the test phase (fifteen trials per condition). If participants failed to achieve this, they were allowed a second practice phase but then had to proceed to the test phase regardless of performance. (In fact, performance was highly accurate in this task.) For each condition, there were two dependent measures (correct trials only): (1) mean latency in ms from the appearance of the stimulus to the release of the press-pad (i.e. reaction time); (2) mean latency in ms from the release of the press-pad to the touching of the stimulus (i.e. movement time). Results were qualitatively similar for the simple and five-choice tasks and so we present only the five-choice data here.

Matching to sample visual search. In this task, participants viewed an abstract pattern, composed of four coloured elements, in the centre of the screen. To obtain the sample pattern, participants were required to hold down the press-pad. Shortly afterwards, two, four or eight similar patterns appeared in a circle of boxes surrounding the original pattern. Participants were required to release the press-pad and touch the single pattern that exactly matched the pattern in the centre of the screen. They were repeatedly encouraged not to release the press-pad until they had decided which was the matching pattern. There were three practice trials followed by eighteen test trials (six trials with two, four and eight patterns, intermixed). There were two dependent measures: (1) response accuracy (the total number of correctly matched patterns out of eighteen, expressed as a percentage); (2) mean latency in ms to release the press-pad on the appearance of the test patterns for correct trials. As accuracy was high (93 % for both age groups) and there was no evidence of a speed–accuracy trade-off, we present only the latency data here.

Data analysis

Experimenters at one of the centres testing older adults (Grenoble, France) terminated the SWM task after completing the six-box condition, thereby omitting the eight-box condition. The main analyses of SWM data are therefore based on reduced numbers of older adults (108 rather than 199). In addition, the total number of participants for each measure was not always 387 because of the very occasional early termination either of the CANTAB session or of a CANTAB task within a session.

Data were analysed using SPSS statistical software package version 11.0 (SPSS Inc., Chicago, IL, USA). Baseline measures (see Table 1) were entered into two-way ANOVA with age group (younger *v.* older) and (subsequent) treatment (0, 15 and 30 mg/d) as between-subjects factors. The main analyses (i.e. Zn and cognitive measures) were three-way repeated measures ANOVA with age group (younger *v.* older) and treatment (0, 15 and 30 mg/d) as between-subjects factors, and time (0, 3 and 6 months) as a within-subjects factor. (Initial analyses included centre (four levels: Coleraine; Clermont-Ferrand; Rome; Grenoble) as a between-subjects factor. These revealed few centre effects or interactions involving centre that could not be attributed instead to differences in age between participants tested in Coleraine and Clermont-Ferrand (younger) *v.* Rome and Grenoble (older). Therefore, data from centres testing participants of the same age were combined in all the reported analyses.) Where there was evidence of departure from the sphericity assumption, Greenhouse–Geisser corrections are reported. In addition, correlations were calculated between Zn measures (intake and blood and urine levels) and cognitive measures (MMSE and CANTAB scores) at baseline (i.e. before supplementation), with age partialled out, separately for males and females.

Results

Baseline measures

The younger group was on average approximately 12·5 years younger than the older group but age did not differ significantly between participants assigned to the three different treatments ($F < 1$), and there was no age × treatment interaction ($F < 1$). For MMSE, there was no effect of age group ($F(1, 381)$ 2·77; $P > 0·05$), or of treatment ($F(2, 381)$ 1·90; $P > 0·1$), and no interaction ($F(2, 381)$ 1·74; $P > 0·1$). There were also no significant effects for geriatric depression scale: age ($F < 1$); treatment ($F(2, 381)$ 2·90; $P > 0·05$); age × treatment ($F(2, 381)$ 1·05; $P > 0·1$). The ANOVA on Zn intake revealed no effect of age group ($F(1, 376)$ 2·59; $P > 0·1$), or of treatment ($F(2, 376)$ 1·13; $P > 0·1$), and no interaction ($F(2, 376)$ 1·91; $P > 0·1$). For serum Zn, there were no significant effects: age ($F(1, 379)$ 1·94; $P > 0·1$); treatment ($F < 1$); age × treatment ($F < 1$). Erythrocyte Zn levels were significantly higher in younger than in older adults ($F(1, 369)$ 13·46; $P < 0·001$); there was no effect of treatment ($F < 1$), and no interaction ($F(2, 369)$ 1·76; $P > 0·1$). Urinary Zn levels were significantly lower in younger than in older adults ($F(1, 376)$ 37·09; $P < 0·001$). There was also a significant effect of treatment ($F(2, 376)$ 3·23; $P < 0·05$); *post hoc* Scheffé tests revealed that only the difference between the 0 and 30 mg/d groups reached significance ($P < 0·05$). There was no age × treatment interaction ($F(2, 376)$ 1·34; $P > 0·1$). In summary, participants in both age groups were reasonably well matched across the three treatments on measures at baseline (Table 1). Younger adults differed from older adults only in terms of erythrocyte (higher) and urinary (lower) Zn levels.

Zinc measures

For both serum and urinary Zn, there were highly significant two-way interactions between treatment and time ($F(4, 758)$ 16·95, $P < 0·001$; $F(3·6, 663)$ 12·43, $P < 0·001$, respectively). Changes between baseline (i.e. month 0) and months 3 and 6 (which were themselves similar) for serum Zn were as follows: the placebo group dropped by 0·25 μmol/l, the 15 mg/d group increased by 1·02 μmol/l, and the 30 mg/d group increased by 2·12 μmol/l. Corresponding changes for urinary Zn were a drop of 0·10 μmol/mmol creatinine (placebo), an increase of 0·13 μmol/mmol creatinine (15 mg/d) and an increase of 0·44 μmol/mmol creatinine (30 mg/d). The trends for erythrocyte Zn were similar ($-3·15$, $+7·14$ and $+8·16$ μmol/l, respectively), but the treatment × time interaction did not reach significance in this case ($F(4, 738)$ 1·79; $P > 0·1$). These data demonstrate significant biological consequences of Zn supplementation and provide confirmation of the participants' compliance with the study's requirements. For all three measures, the three-way interaction between age, treatment and time was not significant (all $P > 0·1$), indicating that younger and older adults reacted similarly in biological terms to the Zn supplementation.

Cognitive measures

Zinc–cognition correlations at baseline. Out of seventy-two correlations, only three reached significance at $P < 0·05$ (r 0·187 between serum Zn and PRM latency for males; r −0·155 between erythrocyte Zn and PRM latency for males; r 0·164 between five-choice reaction time and erythrocyte Zn for females) and only one at $P < 0·01$ (r 0·206 between serum Zn and five-choice reaction time for males). Thus there was no strong evidence of any consistent relationships between Zn intake or blood and urine levels of Zn and cognitive measures at baseline.

Age × treatment × time analysis of variance. First, for each dependent measure, there was a highly significant effect of age group (all $P < 0·001$). Younger adults were more accurate (PRM, SWM, spatial span), faster (PRM, MTS, five-choice reaction time, five-choice movement time) and more efficient (SWM strategy) than older adults (for overall means, see Table 2).

Second, there were significant main effects of time for PRM accuracy ($F(2, 760)$ 3·67; $P = 0·026$), PRM latency ($F(1·8, 681)$ 29·07; $P < 0·001$), SWM total errors ($F(1·9, 561)$ 35·21; $P < 0·001$), SWM strategy ($F(2, 580)$ 9·52; $P < 0·001$) and MTS latency ($F(2, 736)$ 15·49; $P < 0·001$), mostly reflecting improvements with practice (see Fig. 1). These differed significantly between younger and older adults (i.e. age × time interactions) for PRM accuracy ($F(2, 760)$ 3·09; $P = 0·046$), SWM total errors ($F(1·9, 561)$ 6·50; $P = 0·002$) and MTS latency ($F(2, 736)$ 4·50; $P = 0·012$). For PRM, accuracy fell slightly between months 0 and 3 for younger adults only (88, 86 and 86 % for months 0, 3, and 6 for younger adults; 82 % for months 0, 3 and 6 for older adults). PRM latency decreased from 2703 to 2462 to 2382 ms from months 0 to 3 to 6. SWM errors decreased over time, but more so for older adults (an overall drop of twelve errors over 6 months) than for younger adults (a drop of six). SWM strategy improved with practice (35·5, 35·1 and 34·4 for months 0, 3 and 6). Finally, MTS latency decreased over time, particularly for younger adults (an overall decrease of 408 ms from months 0–6) compared with older adults (a decrease of 134 ms).

Third, there were significant treatment × time interactions for SWM errors ($F(3·9, 561)$ 2·74; $P = 0·030$) and MTS latency

Table 2. Overall measures for younger and older adults on tests from the Cambridge Automated Neuropsychological Test Battery

(Mean values with their standard errors)

		Younger***		Older***	
Test	Measure	Mean	SE	Mean	SE
PRM	Accuracy (% correct)	86·80	0·60	82·02	0·58
PRM	Mean correct latency (ms)	2293·30	48·93	2738·36	47·78
SWM	Total errors	32·98	1·12	40·01‡	1·47
SWM	Strategy†	34·24	0·29	35·81‡	0·39
SSP	Span length	5·24	0·05	4·62	0·05
MTS	Mean correct latency (ms)	2781·47	78·48	3878·98	77·02
Five-choice reaction time	Mean correct latency (ms)	387·67	5·82	442·31	5·70
Five-choice movement time	Mean correct latency (ms)	465·35	7·66	584·00	7·48

PRM, pattern recognition memory; SWM, spatial working memory; SSP, spatial span; MTS, matching to sample visual search.

*** All differences between younger and older age groups were significantly different ($P<0·001$).

† A higher score indicates a less efficient strategy.

‡ Older adults' data based on 108 participants rather than 199 (for details, see p. 755).

For details of subjects and procedures, see p. 753.

($F(3·9, 736)$ 3·15; $P=0·015$). For SWM errors, the interaction could be attributable to greater improvement from months 0 to 3 for the 15 and 30 mg/d treatments than for placebo and/or to greater improvement from months 3 to 6 for the 0 and 15 mg/d treatments than for the 30 mg/d treatment. Separate ANOVA on months 0–3 and on months 3–6 both revealed significant treatment × time interactions ($P=0·046$ and 0·014, respectively). However, it is not clear from Fig. 1(c) that either the 15 or 30 mg/d treatment produced any substantial or lasting benefit in terms of SWM errors over placebo. Note also that an ANOVA on SWM errors from four and six boxes only (i.e. excluding eight boxes and thereby reinstating the data from older adults tested in Grenoble) produced no treatment × time interaction ($F < 1$).

For MTS latency, the significant interaction was due to greater improvement over the 6 months for the 0 and 30 mg/d treatments than for the 15 mg/d treatment (see Fig. 1(f)). There was also a marginal interaction for SWM strategy ($F(4, 580)$ 2·31; $P=0·057$; Fig. 1(d)), with the 30 mg/d group showing less improvement from 0 to 3 months than the other groups, but this may reflect a ceiling effect as the 30 mg/d group had the best strategy score of the three groups at baseline. Zn supplementation had no influence on PRM, spatial span or five-choice performance. Note that, with a sample size of 387, of whom 257 received supplements, power was sufficiently high ($>0·80$) to detect even a small effect size, given that the critical comparisons (i.e. performance before and after supplementation) were essentially within-subjects.

Finally, there were no other significant effects. In particular, there were no three-way (age × treatment × time) interactions, indicating that the effects of Zn supplementation (such as they were) did not differ significantly between younger and older adults.

Discussion

To summarise the main findings, Zn intake at baseline was similar in younger and older adults (mean ages of 62 and 74 years, respectively) but erythrocyte Zn was higher and urinary Zn was lower in younger adults than in older adults, with no age difference for serum Zn. There were almost no significant associations between either Zn intake or status at baseline and measures of cognitive function. Supplementation was effective in that serum and urinary Zn levels both increased, and more so for the higher dose (30 mg/d) than for the lower dose (15 mg/d), with similar trends for erythrocyte Zn. These biological effects of Zn supplementation did not differ between younger and older adults. For the cognitive measures, younger adults outperformed older adults on all tests. Crucially, however, there were only two significant interactions indicating effects of Zn supplementation on cognitive function, with a beneficial effect (but only at 3 months) of 15 and 30 mg/d for SWM errors, and a detrimental effect of 15 mg/d for MTS latency. Additional analyses not reported here showed that these findings were the same for: (1) males and females; (2) lower and higher functioning individuals (based on MMSE scores); (3) those with no/primary, secondary and tertiary education; (4) individuals with lower and higher Zn intakes and serum levels.

Zn intake was unaffected by age, which is perhaps surprising in view of previous literature (see p. 752). It was slightly higher overall at 10·9 mg/d than in a study of 2974 adults with a mean age of 70 years whose average Zn intake was 9·9 mg/d (Ma & Betts, 2000), and in the Age-Related Eye Disease Study Research Group (2002) study of 3635 adults with a median age of 69 years whose average Zn intake was 9·4 mg/d. RDA for Zn appear to vary somewhat between countries and over time – for example, US RDA can be found of 12 and 15 mg/d for females and males, respectively (Food and Nutrition Board & National Research Council, 1989), and 8 and 11 mg/d (Food and Nutrition Board & Institute of Medicine, 2000). Based on the more recent figures, the percentages of participants in the present study whose Zn intake was less than two-thirds of the RDA were small at 5·3 (females) and 8·8 (males). It seems that, contrary to previous studies (for example, Briefel *et al.* 2000), Zn intake (at least as estimated from analysis of 4 d food diaries) was adequate for the majority of our participants (see also Andriollo-Sanchez *et al.* 2005).

Urinary Zn was higher in older adults than in younger adults, suggesting changes in Zn metabolism with age. Erythrocyte but not serum Zn decreased with age. The overall mean for serum Zn of 13·1 μmol/l was roughly comparable with the mean from the Age-Related Eye Disease Study Research Group (2002) study of 12·7 μmol/l. In the present study, the percentages of participants

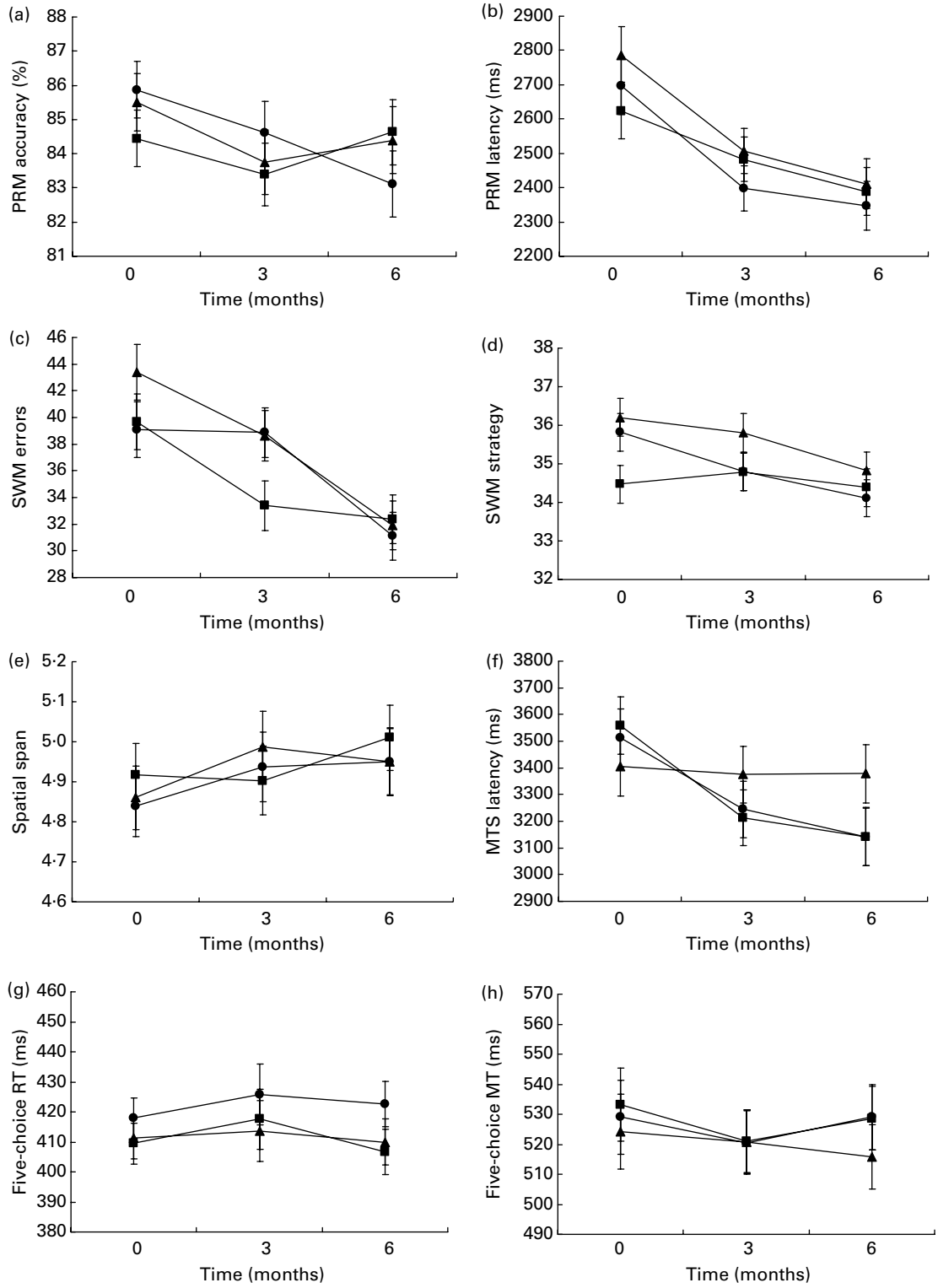

Fig. 1. Mean scores on the Cambridge Automated Neuropsychological Test Battery immediately before Zn supplementation (0 months) and after 3 and 6 months of supplementation for each of three treatments (0 (–●–), 15 (–▲–) and 30 (–■–) mg/d) for (a) pattern recognition memory (PRM) accuracy (% correct), (b) PRM mean correct latency (ms), (c) spatial working memory (SWM) total number of errors, (d) SWM strategy (for details, see p. 755), (e) spatial span, (f) matching to sample visual search (MTS) mean correct latency (ms), (g) five-choice reaction time (RT) mean correct latency (ms) and (h) five-choice movement time (MT) mean correct latency (ms). Data are means, with standard errors represented by vertical bars. For details of subjects and procedures, see p. 753.

with a baseline serum Zn level below the accepted cut-off for Zn deficiency of 10·7 μmol/l (see Andriollo-Sanchez *et al.* 2005) were 2·6 (females) and 2·1 (males). This low prevalence of Zn deficiency contrasts with higher rates in other samples such as hospitalised older adults (for example, Schmuck *et al.* 1996; Pepersack *et al.* 2001).

The complete absence of any relationships between Zn intake and cognitive measures (MMSE and CANTAB scores) at baseline contrasts with the results of Ortega *et al.* (1997). They found a significant, but relatively weak, correlation between Zn intake and MMSE score that accounted for less than 2 % of the variance. The present study employed more sensitive cognitive measures and almost 50 % more participants but failed to replicate the findings of Ortega *et al.* (1997), which may indeed have been due to one of many possible confounding factors noted in their paper.

Age-related deficits were observed on all CANTAB measures, as expected from previous studies (for example, Robbins *et al.* 1994, 1998; Lowe & Rabbitt, 1998; Rabbitt & Lowe, 2000; De Luca *et al.* 2003). There were significant effects of repeated testing for some of the measures, particularly between the first and second testing occasions (months 0 and 3). In one case, the younger adults benefited more from practice than did the older adults, and in another case, the opposite was true (for a discussion of related observations, see Lowe & Rabbitt, 1998).

Importantly, there were few significant effects of Zn supplementation on cognitive function – of the eight measures, one showed a short-lived beneficial effect (but see p. 757) and another showed a detrimental effect of 15 but not 30 mg/d. This paucity of Zn effects was despite significant changes in biological measures (for example, serum Zn) showing at least that participants had successfully taken the supplements as instructed. There may be several explanations for the present results. As already noted, we can rule out the possibility that participants did not comply with the study's requirements or that the amounts of Zn (15 and 30 mg/d) were too low to have any measurable effects. We can also probably discount a lack of power as the explanation (see p. 757). Note further that the cognitive tests employed were sufficiently challenging and sensitive to produce marked age-related deficits. Perhaps more likely is the possibility that the present participants were not sufficiently deficient in Zn (either in terms of intake or serum levels) to gain much benefit from supplements. Certainly, they were a highly selective sample, with 49 % of the initial 842 volunteers being excluded from the study at screening on the basis of cognitive impairment, depression, pathological conditions, medications, and so on. We therefore do not know whether the present results would generalise to the less healthy half of the population who were excluded from the study. Thus it remains possible that the results of Zn supplementation on cognitive function would be more positive in other, more vulnerable, populations (for example, cognitively impaired or hospitalised older adults).

Although the present results revealed evidence of only one beneficial effect of Zn supplements on cognitive function, they also showed only one adverse effect. This is important because older adults may take Zn supplements for non-cognitive reasons – for example, the Age-Related Eye Disease Study Research Group (2001) study showed that antioxidants and Zn supplements reduced the progression of age-related macular degeneration. It is also reassuring in view of the growing availability and use of both fortified foods and dietary supplements.

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