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Original Articles

Zinc for Attention-Deficit/Hyperactivity Disorder: Placebo-Controlled Double-Blind Pilot Trial Alone and Combined with Amphetamine

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Abstract

Objective: To explore effects of zinc supplementation in American children with attention-deficit/hyperactivity disorder (ADHD). Mideastern trials reported significant benefit from 13–40 mg elemental zinc as the sulfate.

Method: We randomly assigned 52 children aged 6–14 with DSM-IV ADHD to zinc supplementation (15 mg every morning [qAM] or two times per day [b.i.d.] as glycinate, $n = 28$) or matched placebo ($n = 24$) for 13 weeks: 8 weeks monotherapy and then 5 weeks with added d-amphetamine (AMPH). AMPH dose was weight-standardized for 2 weeks and then clinically optimized by week 13. Zinc glycinate was chosen as having less gastrointestinal discomfort than sulfate. Hypotheses were that zinc would improve inattention more than placebo by effect size of $d > 0.25$ at 8 weeks; zinc+AMPH would improve ADHD symptoms more than placebo+AMPH by $d > 0.25$, and optimal dose of AMPH with zinc would be 20% lower than with placebo. An interim analysis requested by the National Institute of Mental Health resulted in an increased dosage, so that 20 received 15 mg/day qAM and 8 received 30 mg/day (15 mg b.i.d.)

Results: Only the third hypothesis was upheld: Optimal mg/kg AMPH dose with b.i.d. zinc was 37% lower than with placebo. Other clinical outcomes were equivocal, sometimes favoring zinc, sometimes placebo, but objective neuropsychological measures mostly favored b.i.d. zinc ($d = 0.36$–0.7). Safety tests and adverse events were not different between groups. Copper and iron blood indices were not impaired by 8 weeks of 30 mg/day zinc.

Conclusion: Doses up to 30 mg/day of zinc were safe for at least 8 weeks, but clinical effect was equivocal except for 37% reduction in amphetamine optimal dose with 30 mg/day zinc (not with 15 mg). Possible reasons for difference from mid-eastern reports include endemic diets, population genetics, relative rate of zinc deficiency, difference in background nutrition, insufficient dosage or absorption, or wrong anion (sulfate may be necessary for reported benefit). Dose may be especially important: All visually impressive advantages over placebo appeared only with 15 mg b.i.d. rather than once a day. Future research should use larger doses than 15 mg/day, provide a basic recommended daily allowance/intake multivitamin/mineral supplement for all to standardize background nutrition, select participants for low zinc, and consider the issue of anion interaction.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is characterized by symptoms of inattention, distractibility, overactivity, and impulsivity excessive for developmental age, beginning by age 7, causing impairment in more than one setting, and not better explained by another disorder. The best documented, most successful, and most widely used treatment is stimulant medication (methylphenidate and amphetamine), which shows a robust effect in group data, with placebo-controlled effect sizes (Cohen's *d*) from 0.7 to 1.5 on parent and teacher ratings of attention and behavior. However, the response rate at the individual patient level is often less than satisfactory. Many of those usually counted as responders in the typically quoted response rate of 2/3–3/4 have considerable room for improvement or have nuisance side effects at their optimal dose. Even with the careful medication management algorithm of the National Institute of Mental Health (NIMH) multisite Multimodal Treatment Study of ADHD (the MTA), the rate of excellent response was only 56%, and for the community-treated comparison group it was only 25% (Swanson et al. 2001). Thus, there is considerable room for improvement in stimulant response.

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Zinc is an important cofactor for metabolism relevant to neurotransmitters, prostaglandins, and melatonin, and indirectly affects dopamine metabolism. It is necessary for 100 different metalloenzymes and metal–enzyme complexes (Toren et al. 1996), many of them in the central nervous system. It contributes to structure and function of brain (Black 1998). Specific to ADHD, the dopamine transporter has a zinc binding site that blocks transport (Lepping and Huber 2010). Both animal data (Halas and Sandstead 1975; Sandstead et al. 1977; Golub et al. 1996) and human findings suggest involvement of zinc deficiency in hyperactivity. Human zinc deficiency syndrome includes concentration impairment and jitters (Aggett and Harries 1979).

In ADHD, zinc has been reported significantly ($p < 0.001$) deficient compared with control subjects, though it is not clear how rigorously the diagnoses were made (Bekaroglu et al. 1996; Toren et al. 1996; Kozielec et al. 1994). For example, Bekaroglu et al. (1996) reported mean serum zinc of 60.6 ± 9.9 mcg/dL in 33 boys and 15 girls with ADHD compared with 105.8 ± 13.2 mcg/dL in healthy volunteers (30 boys and 15 girls). Starobrat-Hermelin (1998) found a high rate of magnesium, zinc, iron, copper, and calcium deficiencies in 116 children with ADHD on the basis of serum, red cell, and hair analyses. Bekaroglu et al. (1996) concluded, ''zinc deficiency may play a role in aetiopathogenesis of ADHD.''

There is a possibility of exacerbation of marginal zinc deficiency by drug or other chemical interactions. Ward et al. (1990) found significantly lower zinc in 20 hyperactive boys compared with 20 age-matched control subjects in urine ($p < 0.001$), hair ($p < 0.001$), serum ($p < 0.01$), 24-hour urine ($p < 0.01$), and nails ($p < 0.01$). When 10 hyperactive boys and 10 age-matched control subjects from that sample were challenged with tartrazine-containing commercial beverages, serum and saliva zinc decreased, whereas urine zinc increased in the hyperactive but not control children. In a larger study Ward (1997) found low zinc and iron in a sample of 486 hyperactive children compared with a sample of 172 normal control subjects. When 47 children with a known behavioral reaction to food dye were challenged with 50 mg of dye, their serum levels of zinc went down and urine levels went up compared with age- and sex-matched normal control subjects. In both studies the changes in zinc levels were associated with behavioral deterioration.

Previous reports suggested that optimal stimulant response may depend on adequate zinc nutrition. Arnold et al. (1990) reported a significant correlation of baseline hair zinc with placebo-controlled d-amphetamine response on parent- and teacher-rated Conners' hyperactivity index and hyperactivity factor ($r = 0.52$–0.61; $p = 0.02$–0.047, two-tailed) in 18 boys age 6–12 with ADHD. In further analysis of the same sample (Arnold et al. 2000), a pediatrician familiar with zinc assessments blindly categorized the 18 boys as having good zinc nutrition ($n = 5$), borderline zinc ($n = 5$), or mild/marginal zinc deficiency ($n = 8$) on the basis of hair, urine, and red cell zinc levels. On the Conners' 10-item hyperactivity index, the placebo-controlled effect size of amphetamine was $d = 1.37$ in the presence of adequate zinc nutrition but only $d = 0.55$ (medium) in the presence of mild/marginal zinc deficiency. Akhondzadeh et al. (2004) examined zinc supplementation of methylphenidate (MPH) response in Iranian children with ADHD. Forty-four children aged 5–11 treated with methylphenidate 1 mg/kg per day in 2 divided doses were randomly assigned to zinc sulfate 50 mg/day containing about 13 mg elemental zinc ($n = 22$) versus placebo ($n = 22$) for 6 weeks. On the DuPaul ADHD rating scale, those assigned to MPH plus supplemental zinc improved significantly more than those assigned to MPH plus placebo by parent ($p < 0.05$) and teacher ($p = 0.04$) ratings.

Bilici et al. (2004) reported a trial of zinc supplementation alone as treatment for ADHD, in a Turkish sample. It was flawed by a very high dropout rate (207/400) after randomization as well as during a lead-in phase: Only 193 of the 618 consented eligible participants completed the trial. Those randomized to zinc supplementation received zinc sulfate 150 mg/day (containing about 40 mg elemental zinc) for 12 weeks, a rather high dose. After 12 weeks' treatment, the 46-item clinician-rated ADHD Scale showed that the supplemented group ($n = 95$ completers, 202 total) improved by 25.4 ± 9.7 (about a 1/3 decrease in score) compared with the placebo group ($n = 98$ completers, 198 total), which improved by only 12.7 ± 5.4 ($p = 0.002$). The significant difference was due to the hyperactivity, impulsivity, and impaired-socialization subscales, with no effect on the attention-deficit subscale. Those who were older and had higher body mass index and lower zinc and essential fatty acid levels were most likely to benefit. Not all outcome measures were significant, and it is not clear whether the cited data are for completers or totals. In another Turkish trial (Uckardes et al. 2009) 218 third-grade children were randomized to placebo or 15 mg elemental zinc as a syrup. A subgroup with mothers with a low level of education showed significant reductions in attention deficit, hyperactivity, and oppositional behavior with zinc but not with placebo by both parent and teacher scales. Because of differences in endemic diet, it is not clear how applicable such Mideastern findings are to American children with ADHD.

The pilot study reported here was designed to answer the following questions:

1. Compared with matched placebo, by how large an effect size, if any, does zinc supplementation alone significantly improve ADHD symptoms over an 8-week period?
2. By how large an effect size, if any, does double-blind zinc supplementation plus open amphetamine yield better behavioral response on parent- and teacher-rated *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) (American Psychiatric Association 1994) ADHD symptoms than does zinc-matched placebo plus open amphetamine?
3. Does preliminary zinc supplementation result in openly titrated amphetamine dose optimization at lower doses than does preliminary zinc-matched placebo?

The descriptive data for a number of measures were also heuristically examined.

Methods

Three tightly linked studies (actually phases of the same study) used the same participants and the same initial randomization to 13 weeks of double-blind zinc versus placebo. Participants were consented for all three phases (and an open-label extension) in a single package using documents and procedures approved by the local Institutional Review Board (IRB).

Phase 1, lasting 8 weeks, addressed question 1

After baseline zinc tissue measures, parent and teacher behavior ratings, Clinical Global Impressions (CGI), physical exam, parent and child children's depression inventory, and neuropsychological cognitive-motor testing, children aged 6–14 years with carefully diagnosed ADHD were randomly assigned to either zinc supplementation (15 mg/day as the glycinate) or zinc-matched placebo, stratified by serum zinc level. Bales et al. (1994) and Thomas et al. (1992) have reported that in cases of moderate zinc

deficiency, zinc functional indicators can be normalized in a week or two of zinc supplementation. In this exploratory study we supplemented for 8 weeks before final measures of clinical effect in phase 1. Behavior ratings by parent and teacher, CGI, and neuropsychological cognitive-motor assessments were repeated at 8 weeks and the behavior ratings and CGI at alternate weeks between to check for time course. All participants were asked at baseline to continue with their usual diet throughout the studies. The outcomes were the effect size of the contrast between zinc-supplemented and placebo-supplemented groups on the clinical and cognitive measures. An effect size of $d \geq 0.25$ was hypothesized.

Phase 2, lasting 2 weeks, addressed question 2

This phase added open-label fixed-dose amphetamine to the double-blind zinc and placebo. The amphetamine dose was based on weight. Behavior ratings by parent and teacher, CGI, and cognitive-motor testing were done at the end of the 2 weeks. Cognitive testing took place 45–240 minutes following the daily dose of extended-release amphetamine. An effect size of $d \geq 0.25$ was hypothesized.

Phase 3, lasting 3 weeks, addressed questions 2 and 3

The double-blind zinc and placebo were continued, whereas the open amphetamine, administered as fixed-dose in phase 2, was openly titrated to optimal clinical effect by close phone monitoring and faxed or phoned parent and teacher ratings. The parent and teacher ratings, CGI, and cognitive testing were repeated at the end of the 3 weeks, but the main outcome for this phase was the contrast in optimized doses for the two double-blind zinc groups. We hypothesized that the amphetamine dose in the zinc group would be at least 20% lower than in the placebo group.

Open extension

In fairness to let every participant try the zinc supplementation, an 8-week open extension with both zinc and continued stimulant was added to phase 3. The blind was not broken until completion of the open extension, so it was possible to compare 8 weeks of zinc supplementation to 21 weeks in a double-blind fashion. The same assessment battery as at week 10 was collected at the end of the extension (week 21), except that teacher ratings were not available if the open extension ran past the school year.

Viewed as a total package, a parallel-group randomized clinical trial of zinc supplementation versus placebo had three main phases: Without medication, with open fixed-dose amphetamine, and with openly titrated amphetamine, and was followed by an open extension of zinc with amphetamine.

Zinc status for purposes of stratification at baseline. Those with serum zinc levels of 79 mcg/dL or lower were classified as having low zinc and those above that as having high zinc for purpose of stratification. This crude classification was not used for moderator analysis; it was only a way of roughly balancing the zinc nutritional status between the randomized groups.

Participants

Fifty-two children and adolescents aged 6–14 years inclusive were recruited from the university child psychiatry clinic, advertisements, flyers, letters to professionals, a Web site, and a waiting list of families who want to be notified about ADHD treatment studies. All participants had a DSM-IV diagnosis of ADHD, inattentive or combined type, by Childen's Interview for Psychiatric Syndromes—parent version (P-ChIPS) and clinical DSM-IV assessment by a child and adolescent psychiatrist, with clinical review of P-ChIPS findings for such co-morbid diagnoses as oppositional-defiant disorder (ODD), conduct disorder, anxiety, and depression or other mood disorder. Any co-morbid diagnosis that required psychopharmacotherapy other than catecholaminergic stimulant was excluded. The only other exclusions were current zinc supplementation or anything that would interfere with assessments or study drug or contraindicate study drug (including other diagnoses that contraindicate study drug). States of infection or other inflammation required postponement of entry until the inflammation cleared because of the interaction of inflammation with zinc levels.

Dosage and dosage forms: treatment strategy

The initial dose of zinc was 15 mg/day, an amount that assured a recommended daily allowance/intake, but not enough to be toxic even if the diet was already adequate. This was approximately the same amount of elemental zinc used by Akhondzadeh et al. (2004) but only 3/8 of that used by Bilici et al. (2004). For the last dozen participants (8 taking zinc) this was increased to 15 mg two times per day [b.i.d.] after an interim analysis suggested that more was needed. We used the glycinate rather than sulfate to avoid the gastrointestinal discomfort reported with the sulfate. The zinc capsules (15 mg available zinc) and matching zinc placebo were donated by Albion Laboratories (Clearfield, UT), which makes a Zn-glycine that one of the co-investigators (R.A.D.) had found effective at raising plasma zinc and increasing activities for plasma 5′-nucleotidase (Blostein-Fujii et al. 1997; Best and DiSilvestro 2001; and unpublished results). Although glycine in large doses might have a behavioral effect in its own right, the amount of glycine included in the zinc glycinate preparation was negligible.

The dose of amphetamine for the fixed-dose phase 2 was 5–15 mg extended-release daily generic d-amphetamine spansules (in the morning) based on weight as follows—$\leq$25 kg: 5 mg/day; 25–45 kg: 10 mg/day; >45 kg: 15 mg/day.

The dose of amphetamine for phase 3 was titrated clinically with information from parent and teacher about response and side effects, using faxed and/or phone-interview scales. The goal was to have all of the 18 DSM-IV ADHD symptoms rated 0 or 1 (just a little) by parent and teacher on the 0–3 metric. When needed, supplemental immediate release dextroamphetamine was given in either the morning or afternoon/evening to achieve the optimal dose and duration of effect for each participant.

Measures

Childen's Interview for Psychiatric Syndromes—parent version. The diagnosis of ADHD and any co-morbid diagnoses such as ODD, conduct disorder, anxiety, depression, other mood disorder, or Tourette's was made by the P-ChIPS (Weller), confirmed by clinical review by a child psychiatrist. For diagnosis, a severity criterion also had to be met (see below).

Parent and teacher behavioral ratings

Parent and teacher ratings of attention, impulsivity, and hyperactivity were obtained every 2 weeks from baseline through week 10 (end of phase 2), weekly during phase 3 (to 13 weeks) and twice during the 8-week compassionate open extension, using the ADHD checklist of 18 DSM-IV ADHD symptoms rated on a 0–3 metric

from not at all to very much, also known as the SNAP-IV (Swanson 1992). The primary behavioral outcome variable for phase 1 was the mean item rating of the 9 inattentive symptoms by parent and teacher, and for phase 2 the item mean of all 18 ADHD symptoms by parent and teacher. In addition, at baseline, week 8 (end of phase 1), week 10 (end of phase 2), and week 13 (end of phase 3), we obtained the 8 DSM-IV ODD items on the same metric as an additional secondary measure.

At baseline, the Conners' Parent Rating Scale-Revised, long version (Conners 2001) obtained a broad profile of the participants. This scale includes the SNAP-IV, but also has many other factors tapping co-morbid pathology. This was repeated at 8 weeks, at 10 weeks, and at 13 weeks.

Cognitive-motor neuropsychological test battery

The following tests were used in this order. All are fully automated and provide an unbiased assessment of the participant's performance (Aman 1991).

1. Short-Term Recognition Memory task (Sprague and Sleator 1977). In this task, the participants are required to look at arrays of three or nine cartoon pictures and try to remember them. After each array is shown, the child is presented with a single picture and she/he must decide whether that was a member of the preceding array by pressing a ''Same'' or ''Different'' switch. Two dependent measures are recorded: Mean accuracy and mean response speed.
2. Continuous Performance Task 11 (Conners 2001). The child is told to respond by pressing the space bar whenever she/he sees any non-X and to refrain from responding when she/he sees the ''X.'' The test lasts for about 14 minutes with a variable delay (1–4 seconds) between stimuli. Dependent variables include errors of omission (failures to respond to an non-X), errors of commission (responses to the X), mean response time, and variability of response time (coefficient of variation).
3. Seat Activity. While children were tested, their seat activity was measured unobtrusively, using a ''wiggle'' seat developed by Sprague and Toppe (1966). A slight tilt of the seat activates a microswitch, and activity is recorded cumulatively by the computer.

Clinical Global Impressions

A secondary clinical outcome measure was the CGI (Psychopharmacology Bulletin 1985), a 7-point clinician scale of severity (CGI-S) and improvement (CGI-I). It was done at baseline (severity only) and at each subsequent visit (severity and improvement).

Zinc assessment

Zinc nutritional status was assessed at baseline and at the end of phase 1. Because no single index is considered definitive for zinc status, we assessed it in three ways. The serum zinc was used for stratification purposes, but a composite unitary index of all three measures was used for data analytic purposes. The three tests in detail are these:

1. Serum zinc was measured by atomic absorption spectrometry (e.g., Bales et al. 1994; Blostein-Fujii et al. 1997). Although plasma/serum is the traditional tissue used for assessing Zn status (Thompson 1991), it has two drawbacks: (i) values do not always fall rapidly in response to small changes in zinc status; (ii) values can be affected by factors other than zinc status. Nonetheless, plasma/serum zinc readings are still considered a starting point to evaluating zinc status.
2. Plasma 5′-nucleotidase activity was assayed by a sensitive method (Bales et al. 1994; Blostein-Fujii et al. 1997; Joung and DiSilvestro 1997) where activity is measured by a kinetic, spectrophotometric method described by Bertrand and Buret (1982).
3. The third assay was erythrocyte metallothionein. This provides an assessment of zinc status in which values do not fluctuate with short-term temporarily high zinc intake (Thompson 1991). In addition, metallothionein values have much wider ranges than do values for 5′-nucleotidase activities. Thus, drastic changes in zinc status over time may produce larger percent changes for metallothionein values as compared with 5′-nucleotidase activities. On the other hand, the latter values can change more quickly than the former with small changes in zinc status. Thus, both measurements are valuable. Metallothionein was measured by an immunoassay developed by Garvey et al. (1982), and used by RAD in a previous human study (Bales et al. 1994). Assay sensitivity is good enough to measure erythrocyte metallothionein at levels well below 5% of normal. Antibody was made in rabbits using rat liver metallothionein purified by the method of Garvey et al. (1982). The protein was homogenized by polyacrylamide gel electrophoresis and the resulting antibody gave one precipitin band with rat liver extracts on Ouchterlony.

Dietary evaluation

The Kid's Food Questionnaire (Wakimoto and Block 2001) was used to quantify typical dietary intake, including zinc intake, at baseline and week 8 (end of phase 1), as a compliance check on the instruction not to alter diet during the study, and to estimate customary zinc intake. The questionnaire asks the respondent to indicate how many times a week or month the child eats a given food and to describe the size of the usual serving either as a number, or for nonunitary foods, as different photos representing various amounts of food, relative to a standard serving. Portion sizes, numbers, and serving sizes were entered into the Block Dietary Data Systems (Berkeley, CA), which multiplies these by the nutrients contained in each food or food group and arrives at an estimated nutrient intake. The Block Dietary Data Systems is based on dietary data collection and a nutrient calculation procedure originally developed for the Lipid Research Clinics and the Multiple Risk Factor Intervention Trial. Data relevant to reliability and convergent validity have been reported by several users of food frequency questionnaires (Eck et al. 1991; Hunter et al. 1992; Stein et al. 1992; Wakimoto and Block 2001).

Depressive symptoms evaluation

Although major depression requiring pharmacotherapy excluded applicants from the study, many of the participants, as expected in a representative ADHD sample, had subclinical depressive symptoms or even diagnoses of mild depression. Since zinc has been reported low in depression as well as ADHD (Maes et al. 1999), it was conceivable that any benefit accruing from zinc might be due to improvement of depression rather than ADHD symptoms. Aside from this confounding effect, it was heuristically

Table 1. Means (Standard Deviation) of Zinc Blood Levels for the First 28 Participants

	Plasma zinc (μg/mL)		*5′-Nucleotidase (units/L)*		*Metallothionein (μg/mg Hb)*	
	Baseline	*Week 8*	*Baseline*	*Week 8*	*Baseline*	*Week 8*
Zinc	0.71 (0.11)	0.69 (0.13)	5.55 (1.08)	5.40 (0.92)	163.4 (51.2)	168.9 (58.64)
Placebo	0.68 (0.17)	0,69 (0.17)	5.76 (1.06)	5.86 (0.92)	148.2 (36.4)	150.9 (30.8)

of interest to see what happens to depressive symptoms with zinc supplementation. Therefore, the parent and child children's depression inventory (Kovacs 1992; Fristad et al. 1997) was administered at screening/baseline, 8, 10, 13, and 21 weeks to track depressive symptoms.

Determination of zinc nutritional status. The three zinc measures were composited for the explorations involving zinc levels in the following way: For each measure at baseline, the sample mean ($n \geq 52$) and standard deviation (SD) were determined. Then the z-score was determined for each participant on each measure at each time (baseline and 8 weeks), using the sample baseline mean and SD as the reference. The three z-scores for each participant at each time were then averaged to define that participant's zinc status at that time (referred to as ''zinc level''). Midway through the study, we started 24-hour urine collections for zinc assays.

Data analysis

The main goal of this pilot study was to decide whether a larger study is warranted. Therefore, the emphasis was on descriptive statistics. Effect size (d) is defined as the ratio between the difference in means between two groups and the SD ($d = [\text{mean } 1 - \text{mean } 2]/\text{SD}$). Thus, the mean differences and SDs comparing the two groups (zinc vs. zinc-matched placebo, both with [phases 2 and 3] and without [phase 1] amphetamine) were calculated for each outcome variable and reported in tabular form, along with baseline and endpoint means. The effect sizes of the difference in change scores were calculated and shown in a table. From phase 3,

Table 2. Interim Analysis of Outcomes

		Adjusted mean by treatment group			
Measure	*Assess points week*	*Placebo mean,* n = *15*	*Zinc mean,* n = *13*	*Root MSE*	*Effect size (ES) (Cohen's* d)
Teacher-rated 9 inattentive symptoms[a] ↓	8	1.588	1.427	0.442	−0.362
	10	1.692	1.485	0.580	−0.357
	13	1.054	0.871	0.463	−0.398
Parent-rated 9 inattentive symptoms[a] ↓	8	1.861	1.915	0.486	0.111
	10	1.556	1.883	0.538	0.610
	13	1.122	1.158	0.466	0.077
Teacher-rated all 18 ADHD symptoms[b] ↓	8	1.590	1.401	0.340	−0.556
	10	1.560	1.272	0.523	−0.547
	13	0.951	0.608	0.482	−0.712
Parent-rated All 18 ADHD symptoms[b] ↓	8	1.731	1.849	0.430	0.280
	10	1.415	1.667	0.488	0.520
	13	0.985	1.060	0.475	0.157
CPT omission errors ↓	8	31.636	33.728	23.547	0.090
	10	26.432	40.032	57.372	0.240
	13	16.382	19.138	18.698	0.148
CPT commission errors ↓	8	21.478	21.526	4.766	0.010
	10	21.196	19.038	7.706	−0.280
	13	20.542	20.035	5.278	−0.096
MFF accuracy ↑	8	77.438	75.070	8.022	−0.290
	10	83.617	78.143	9.432	−0.580
	13	83.355	82.252	9.487	−0.116
Seat movement ↓	8	0.337	0.224	0.397	−0.378
	10	0.462	0.277	0.592	−0.315
	13	0.377	0.152	0.233	−0.990
Dose[c] ↓	13	10.447	13.897	5.406	0.639

Polarity indicated by ↑ for higher score better (positive ES favors zinc) and ↓ for lower score better (negative ES favors zinc). Means adjusted for baseline score, sex, ADHD subtype, and weight.

[a]Primary outcome for phase 1 at 8 weeks.

[b]Primary outcome for phase 2 at 10 weeks.

[c]Primary outcome for phase 3.

ADHD = attention-deficit/hyperactivity disorder; CPT = Conners' Continuous Performance Test; MMF = Matching to Familiar Figures (short-term memory); MSE = mean square error.

Table 3. Means and (Standard Deviations) for Zinc Pharmacokinetic Study

Time (hours)	*Plasma_Zn (μg/mL)*	*RBC_Zn (μg/g Hb)*	*Urine_Zn (μg/mL)*
ADHD patients (n = *5)*			
0	0.78 (0.09)	12.63 (7.2)	
1	0.94 (0.17)	14.05 (9.51)	0.74 (0.32)
2	0.83 (0.15)	10.19 (7.1)	0.52 (0.26)
4	0.76 (0.13)	9.43 (8.51)	0.23 (0.1)
5	0.72 (0.21)	10.75 (7.68)	0.22 (0.1)
7	0.67 (0.14)	10.28 (8.61)	0.25 (0.22)
9	0.64 (0.16)	10.1 (7.87)	0.15 (0.09)
Normal control subjects (n = *2)*			
0	0.79 (0.01)	5.92 (2.29)	
1	1.03 (0.21)	6.75 (1.25)	0.79 (0.09)
2	0.9 (0.22)	3.98 (5.57)	0.77 (0.13)
4	0.8 (0.11)	1.82 (2.51)	0.22 (0.08)
5	0.63 (0.26)	5.15 (3.65)	0.17 (0.05)
7	0.7 (0.03)	2.81 (3.95)	0.15 (0.04)
9	0.63 (0.02)	2.86 (0.7)	0.11 (0.01)

ADHD = attention-deficit/hyperactivity disorder; Zn = zinc.

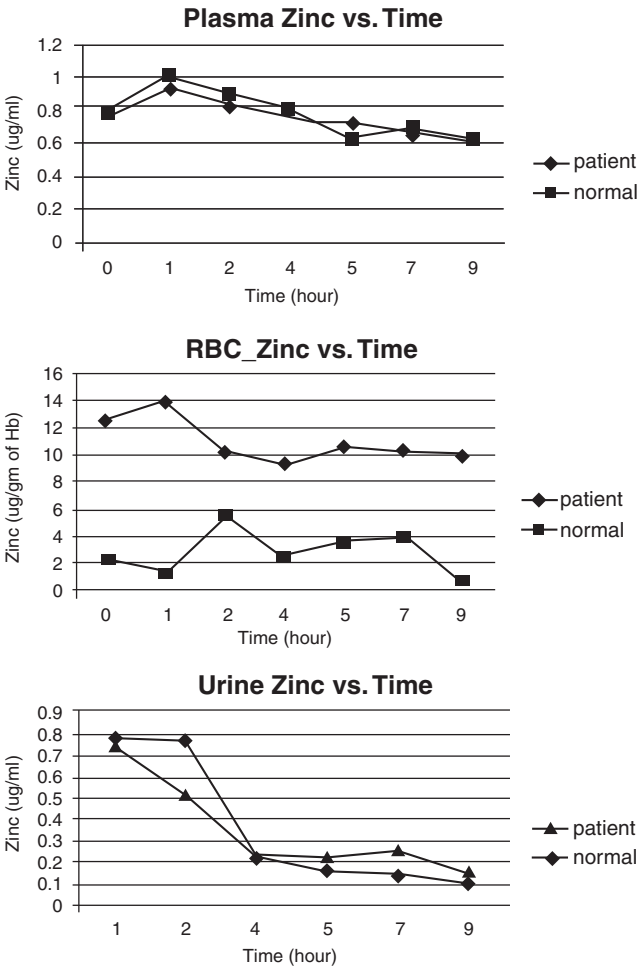

FIG. 1. Pharmacokinetic graphs.

the mean mg/day and mg/kg per day of amphetamine at the end of the open amphetamine titration were calculated for each group (zinc vs. placebo) and compared. Missing data were handled by carrying forward the last observation to the endpoint of a given phase for all participants who enter that phase.

Determination of clinically significant effect size. In the case of such a presumably safe, cheap, and easy treatment, a placebo-controlled effect size of $d = 0.25$ seemed clinically promising enough to warrant further study.

Interim Analysis

We noticed in the first two dozen participants that nothing seemed to be happening beyond what would be expected with placebo. Therefore, an unblinded collaborator checked the changes in blood levels of zinc for the first 28 participants and noted that the random small changes the first 8 weeks appeared in both treatment groups to be what would be expected from placebo (Table 1). Samples of the supplement capsules (both active supplement and placebo) were assayed to make sure they contained the stated amount of zinc, and they did. The amount of zinc in 24-hour urine collections averaged 1.254 μmol/mmol creatinine for those taking zinc and 0.704 for those taking placebo ($p = 0.008$). When four participants taking placebo started open zinc, their 24-hour urine zinc rose from 0.754 to 1.055. Thus, it appeared that some zinc was being absorbed and wasted without correcting the low tissue levels. Therefore, we requested permission from the IRB and NIMH to increase the dose. At this point NIMH requested an interim analysis of clinical outcome data to check more closely on the effects of 15 mg/day before approving an increase.

Results of interim analysis of first 28 participants

No participant dropped out during double-blind phases. The 28 participants in the interim analysis were 13 with zinc and 15 with placebo. Adverse events (AEs) were similar between groups (35 vs. 37). The clinical and cognitive outcome baseline-adjusted means are shown in Table 2.

Summary of primary outcome effect sizes (Cohen's *d*, difference in adjusted means/pooled SD):

- Phase 1 (8 weeks, inattentive Sx): Teacher = −0.36 (favoring zinc), Parent = 0.11 (favoring placebo)
- Phase 2 (10 weeks, all 18 ADHD Sx): Teacher = −0.55 (favoring zinc), Parent = 0.52 (favoring placebo),
- Phase 3 (13-week dose comparison): $d = 0.639$ (lower amphetamine dose with placebo)

Phase 3 main secondary outcome (ADHD Sx): teacher = −0.71 (favoring zinc), parent = 0.16 (favoring placebo).

Conclusions from interim analysis

The significant increase in urine zinc compared with placebo, coupled with the failure of three different blood tests to show a rise in zinc, suggests that administered zinc is immediately excreted. The fact that teacher ratings show a medium effect size favoring zinc over placebo, whereas parent ratings if anything tend the opposite direction are reminiscent of the effect of immediate-release stimulant given only during school hours, with after-school wear-off and withdrawal irritability resulting in a similar pattern of difference in teacher and parent observations. These findings suggested this hypothesis: Children with ADHD may have zinc-wasting metabolism such that zinc administered in the morning is excreted by midafternoon, but while it is in the system it improves

ADHD symptoms—in other words, it may act like an immediate-release stimulant.

Modification of dosing, safety measures, and final analysis based on interim analysis

To explore this hypothesis (rapid excretion with benefit while in the system), NIMH and the IRB approved twice daily zinc dosing for the last 12 participants and a zinc pharmacokinetic study. Therefore, the sample has three groupings for some analyses: Placebo ($n = 24$), 15 mg zinc in the morning ($n = 20$), and 15 mg zinc in the morning and afternoon ($n = 8$). The latter dosing allowed parent ratings in the evening after a more recent dose so that parents would have a chance to report the same effects as teachers did. There was no expectation that placebo would differ appreciably between morning and b.i.d. dosing, so all placebo cases were pooled for analyses. Because the last few participants lacked complete teacher ratings (due to end of school year) and because the morning dose remained the same, all teacher ratings for both zinc groups were pooled. For a closer scrutiny of safety at the higher daily dose, copper and iron assays were added to the zinc assays to check for possible interference with copper and iron absorption.

Zinc Pharmacokinetic Pilot Study

Because of the questions about absorption of zinc from the supplement used and about the fleeting effect suggested by the interim analysis, a small pharmacokinetic study was done to examine time-course blood level changes in ADHD and normal children from the study supplement. In the General Clinical Research Unit, children age 6–12 had their blood and urine zinc tested seven times over a course of 9 hours. They arrived fasting and after the initial blood draw took 15 mg zinc as the glycinate, the same formulation and batch as used in the pilot trial. Low-zinc breakfast, snacks, and lunch were provided. The results are shown in Table 3

Table 4. Participant Characteristics

	Group		
Demographics and clinical features	*Placebo,* n = *24*	*Zinc_1 (qAM),* n = *20*	*Zinc_2 (b.i.d.),* n = *8*
Boys, *n* (%)	20 (83.3%)	15 (75.0%)	8 (100.0%)
Age, mean (SD)	10.24 (2.69)	9.61 (3.36)	8.89 (2.31)
Weight, mean (SD)	36.71 (12.1)	36.75 (13.75)	32.35 (8.47)
ADHD, inattentive type, $n = 14$ (%)	7 (29%)	3 (15%)	4 (50%)
ADHD, combined type, $n = 38$ (%)	17 (71%)	17 (85%)	4 (50%)
ODD/CD[a], $n = 34$ (65%)	18 (75%)	12 (60%)	4 (50%)
Adjustment D/O wth depression[b], $n = 9$	4 (17%)	4 (20%)	1 (13%)
Elimination D/O $n = 4$ (8%)	0	2 (10%)	2 (25%)
Phonological D/O, $n = 5$ (10%)	2 (8%)	1 (5%)	2 (25%)
Learning D/O, $n = 10$ (19%)	5 (21%)	4 (20%)	1 (13%)
Prior ADHD medication, any	13	7	3
Prior amphetamine	6	5	1
Withdrew from stimulant to enter	2	3	0
Race			
African American	2 (8.3%)	3 (15.0%)	3 (37.5%)
Caucasian	22 (91.7%)	15 (75.0%)	5 (62.5%)
Other	0 (0.00%)	2 (10.0%)	0 (0.0%)
Parent marital status			
Single	2 (8.3%)	2 (10.0%)	2 (25.0%)
Married	18 (75.0%)	15 (75.0%)	5 (62.5%)
Divorced	3 (12.5%)	3 (15.0%)	1 (12.5%)
Common law	1 (4.2%)	0 (0.0%)	0 (0.0%)
Family income			
<20,000	1 (4.2%)	4 (20.0%)	3 (37.5%)
20,000–30,000	4 (16.7%)	4 (20.0%)	1 (12.5%)
30,000–50,000	5 (20.8%)	6 (30.0%)	0 (0.0%)
50,000–100,000	11 (45.8%)	4 (20.0%)	3 (37.5%)
>100,000	3 (12.5%)	2 (10.0%)	1 (12.5%)
Mother's education			
Less than high school	2 (8.3%)	0 (0.0%)	1 (12.5%)
High school graduate	3 (12.5%)	7 (35.0%)	0 (0.0%)
Some college	6 (25.0%)	7 (35.0%)	4 (50.0%)
College graduate	12 (50.0%)	5 (25.0%)	2 (25.0%)
Postgraduate	1 (4.17%)	1 (5.0%)	1 (12.5%)

[a]ODD and CD were collapsed because there was only one CD.

[b]Because randomization was balanced by significant depressive symptoms, the category of adjustment D/O with depression was used to categorize those participants. No one had major depression.

ADHD = attention-disorder/hyperactivity disorder; b.i.d. = two times per day; CD = conduct disorder; ODD = oppositional-defiant disorder; SD = standard deviation; D/O = disorder; qAM = in the morning.

Table 5. Blood Levels of Minerals

Minerals	*Time*	*Placebo*	*Zinc_1 (morning only)*	*Zinc_2 (b.i.d.)*
Zinc (mg/mL)	Baseline	0.68 (0.15)	0.71 (0.1)	0.70 (0.11)
	Week 8	0.72 (0.11)	0.70 (0.16)	0.82 (0.24)
	Difference	0.04 (0.16)	−0.01 (0.17)	0.12 (0.33)
5′-nucleotidase (U/L)	Baseline	5.46 (1.08)	5.16 (1.14)	5.33 (1.22)
	Week 8	5.56 (1.02)	5.13 (0.94)	5.02 (1.03)
	Difference	0.11 (0.42)	−0.03 (0.46)	−0.19 (0.41)
RBC_MT (mg/g Hb)	Baseline	63.02 (21.84)	60.21 (18.98)	68.37 (28.21)
	Week 8	62.73 (15.73)	62.41 (22.28)	73.03 (31.06)
	Difference	−0.29 (15.45)	2.20 (18.68)	4.66 (18.64)
RBC_HB (g/mL)	Baseline	0.307 (0.04)	0.32 (0.02)	0.27 (0.04)
	Week 8	0.312 (0.03)	0.34 (0.02)	0.29 (0.03)
	Difference	0.006 (0.03)	0.02 (0.03)	0.02 (0.02)
Ferritin (ng/mL)	Baseline	17.80 (9.57)	18.14 (14.03)	20.98 (7.97)
	Week 8	17.33 (11.94)	15.08 (10.99)	21.10 (8.96)
	Difference	−0.47 (9.42)	−3.06 (10.31)	0.12 (7.34)
Ceruloplasmin	Baseline	0.15 (0.05)	0.146 (0.044)	0.18 (0.06)
	Week 8	0.17 (0.05)	0.153 (0.051)	0.20 (0.09)
	Difference	0.02 (0.06)	0.007 (0.034)	0.02 (0.07)
RBC_SOD (U/mL)	Baseline	3,274.5 (533.9)		3,805.0 (443.4)
	Week 8	3,430.0 (99.0)		3,650.2 (731.5)
	Difference	155.5 (632.9)		−154.8 (621.4)

Zinc_1 was 15 mg zinc as glycinate in the morning daily. Zinc_2 was the same dose twice daily, morning and afternoon/evening.
A positive difference indicates an increase.
b.i.d. = two times per day.

and Figure 1. The serum zinc level did not differ appreciably between the ADHD patients and the normal control subjects: Both the peak, the area under the curve, and the time of the peak appeared almost congruent. In both cases, there was a modest increase at 1 hour after zinc ingestion and then gradual decline over the day. This was matched by a high urinary excretion the first hour or two and then rapid decrease by 4 hours to a flat valley. Although urinary zinc appeared higher at 2 hours in the normal comparison children than in the ADHD patients, the number did not justify attempting a statistical test on the difference. Although the RBC zinc levels appeared higher in the ADHD patients throughout, the small number does not allow testing nor interpretation of that difference.

Results of Full Pilot Randomized Trial

Of 59 children screened, 52 were randomized: 24 to placebo, 20 to zinc in the morning, and 8 to zinc b.i.d. The screen failures were two for anxiety requiring other treatment (in one case pharmacological, the other family therapy and cognitive behavioral therapy), one for chronic tics that might have been exacerbated by the study amphetamine, two for lack of teacher ratings, and two for withdrawal of consent. Characteristics of the sample are shown in Table 4. The severity of ADHD symptoms (parent-rated item mean ± SD of 2.06 ± 0.50 and teacher-rated mean of 2.04 ± 0.49 on the 0–3 scale) was comparable to that reported for other samples characterized as moderately to severely symptomatic, such as the MTA (MTA Cooperative Group 1999), for which parent ratings on the same scale were 1.97 ± 0.64 and teacher ratings 2.11 ± 0.75.

Of the 52 randomized youngsters, 49 completed all double-blind phases and two additional completed the first two double-blind phases, making 51 completing the first two double-blind phases (zinc monotherapy and zinc plus a standard dose of amphetamine). One child, taking zinc b.i.d., dropped out at week 2 because of gastrointestinal discomfort. One child who completed the double-blind phases taking placebo refused the open extension. Of the four total who dropped out (one at week 2, one at week 10, one at week 12, and one at week 13 after completing the double-blind protocol), two were taking placebo and two were taking zinc. Of the four, two (one zinc and one placebo) dropped out because of refusal to take capsules and two (one zinc and one placebo) dropped out for side effects—in the case of the one taking placebo, for amphetamine side effects.

Mineral assays (Table 5) show negligible change in zinc parameters. The red cell metallothionein, a marker of long-term zinc nutrition, did increase about 7% with b.i.d. zinc compared with half that with single-dose zinc and none with placebo; in general, the b.i.d zinc seemed to affect serum level and metallothionein more favorably than either placebo or single-dose zinc. However, this rather nominal tendency was counterbalanced by a reverse nominal tendency for 5′-nucleotidase and might be best characterized as random fluctuations. The safety mineral assays also showed negligible changes. Red cell hemoglobin, ferritin, and ceruloplasmin all showed nominal increases, indicating no interference with iron or copper absorption over the 8-week period, although copper superoxide dismutase, another measure of copper, did show a nominal (4%) decrease over the 8 weeks.

Clinical outcomes (Tables 6A and 6B) showed no definite tendency. On some measures zinc was associated with nominally more improvement, but on others placebo was nominally better. (Because the interim analysis suggested that the morning dose of zinc was responsible for effects rated by teachers and we did not expect an effect on teacher ratings from the afternoon dose, and because so many teacher ratings were missing because of vacations, the two zinc dosing schedules [morning only and b.i.d.] were collapsed for teacher ratings.) Laboratory cognitive-motor outcomes (Table 7)

Table 6A. Means (Standard Deviation) of Clinical Measures: Parents' Ratings

	Baseline			*Week 8*			*Week 10*			*Week 13*			*Week 21*[a]		
Measure	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*
SNAP 9 DSM-IV inattentive Sx	2.38 (0.54)	2.39 (0.39)	2.17 (0.58)	2.12 (0.55)	2.00 (0.58)	1.85 (0.77)	1.68 (0.51)	1.73 (0.74)	1.40 (0.68)	1.23 (0.38)	1.32 (0.7)	0.97 (0.64)	0.98 (0.47)	1.14 (0.76)	0.73 (0.43)
SNAP 9 DSM-IV hyperactive-imp. Sx	1.95 (0.71)	2.13 (0.65)	1.38 (1.13)	1.69 (0.83)	1.84 (0.66)	1.08 (1.08)	1.26 (0.71)	1.49 (0.53)	1.11 (0.88)	0.79 (0.55)	1.06 (0.6)	0.36 (0.61)	0.67 (0.52)	0.85 (0.48)	0.62 (0.98)
SNAP All 18 DSM-IV ADHD symptoms	2.14 (0.52)	2.15 (0.46)	1.66 (0.57)	1.90 (0.63)	1.92 (0.54)	1.47 (0.65)	1.47 (0.51)	1.61 (0.52)	1.26 (0.62)	1.01 (0.38)	1.19 (0.56)	0.67 (0.38)	0.82 (0.44)	0.99 (0.52)	0.67 (0.56)
SNAP ODD DSM-IV symptoms	1.33 (0.59)	1.63 (0.67)	1.12 (0)	1.11 (0.63)	1.49 (0.78)	1.02 (0.81)	0.92 (0.7)	1.22 (0.73)	0.80 (0.55)	0.60 (0.41)	0.87 (0.79)	0.63 (0.32)	0.49 (0.36)	0.85 (0.77)	0.75 (0.96)
Conners parent rating scale															
Overall	107.83 (31.9)	115.85 (30.16)	93.13 (34.53)	95.54 (28.16)	104.95 (33.46)	78.63 (40.09)	65 (28.35)	81 (28.75)	61.63 (36.67)	49.09 (27.47)	59.61 (26.95)	41.63 (19.68)	39.74 (28.44)	52.95 (32.19)	35.43 (28.91)
Global index	1.61 (0.52)	1.71 (0.4)	1.60 (0.78)	1.47 (0.55)	1.62 (0.5)	1.25 (0.68)	1.00 (0.53)	1.35 (0.42)	1.03 (0.64)	0.77 (0.45)	0.97 (0.44)	0.68 (0.6)	0.61 (0.49)	0.76 (0.58)	0.76 (0.57)
Oppositional	1.05 (0.61)	1.28 (0.64)	1.44 (0.79)	1.01 (0.51)	1.20 (0.54)	1.3 (0.86)	0.73 (0.61)	1.09 (0.56)	0.97 (0.69)	0.58 (0.41)	0.74 (0.5)	0.76 (0.71)	0.48 (0.34)	0.61 (0.57)	0.85 (0.88)
Cognitive problems	2.17 (0.41)	1.98 (0.47)	2.18 (0.58)	1.97 (0.49)	1.92 (0.74)	1.90 (0.64)	1.35 (0.57)	1.50 0(0.74)	1.32 (0.82)	1.01 (0.47)	1.15 (0.52)	1.03 (0.71)	0.82 (0.56)	0.89 (0.7)	0.95 (0.7)
Hyperactive	1.55 (0.74)	1.65 (0.7)	1.48 (0.97)	1.40 (0.74)	1.56 (0.62)	1.15 (0.92)	0.88 (0.6)	1.22 (0.61)	0.97 (0.85)	0.61 (0.55)	0.83 (0.48)	0.50 (0.58)	0.49 (0.49)	0.68 (0.48)	0.58 (0.71)
Anxious shy	0.41 (0.53)	0.34 (0.38)	0.56 (0.65)	0.32 (0.49)	0.26 (0.2)	0.45 (0.77)	0.15 (0.25)	0.14 (0.11)	0.29 (0.44)	0.09 (0.2)	0.07 (0.1)	0.29 (0.45)	0.08 (0.14)	0.08 (0.15)	0.13 (0.21)
Perfectionistics	0.50 (0.51)	0.65 (0.48)	0.49 (0.53)	0.38 (0.41)	0.57 (0.42)	0.40 (0.5)	0.31 (0.37)	0.52 (0.31)	0.32 (0.46)	0.27 (0.42)	0.30 (0.24)	0.11 (0.19)	0.19 (0.32)	0.31 (0.28)	0.19 (0.29)
Social problems	0.43 (0.58)	0.71 (0.74)	0.48 (0.32)	0.28 (0.36)	0.55 (0.6)	0.39 (0.31)	0.17 (0.25)	0.38 (0.65)	0.29 (0.37)	0.13 (0.24)	0.26 (0.57)	0.20 (0.32)	0.11 (0.23)	0.22 (0.4)	0.17 (0.24)
Psychosomatic	0.63 (0.65)	0.37 (0.38)	0.59 (0.58)	0.56 (0.6)	0.32 (0.38)	0.35 (0.36)	0.31 (0.39)	0.19 (0.31)	0.19 (0.29)	0.30 (0.39)	0.19 (0.23)	0.15 (0.16)	0.26 (0.38)	0.18 (0.34)	0.11 (0.2)
ADHD index	2.26 (0.49)	2.17 (0.44)	2.19 (0.46)	2.05 (0.53)	2.11 (0.55)	1.90 (0.67)	1.35 (0.55)	1.63 (0.58)	1.33 (0.62)	1.00 (0.49)	1.25 (0.51)	1.04 (0.71)	0.82 (0.64)	0.97 (0.56)	1.04 (0.71)
Restless/impulsive	1.89 (0.58)	2.08 (0.51)	1.70 (0.85)	1.70 (0.65)	1.93 (0.49)	1.42 (0.73)	1.17 (0.61)	1.50 (0.49)	1.15 (0.7)	0.83 (0.52)	1.14 (0.54)	0.73 (0.68)	0.68 (0.58)	0.88 (0.62)	0.77 (0.55)
Emotional lability	0.93 (0.72)	0.83 (0.52)	1.27 (0.87)	0.92 (0.73)	0.92 (0.71)	0.90 (0.79)	0.64 (0.59)	1.00 (0.7)	0.79 (0.67)	0.57 (0.52)	0.56 (0.52)	0.59 (0.58)	0.43 (0.41)	0.49 (0.55)	0.67 (0.69)
9 DSM-IV Inattentive Sx	2.38 (0.51)	2.15 (0.51)	2.16 (0.63)	2.13 (0.5)	2.03 (0.63)	1.90 (0.75)	1.48 (0.58)	1.64 (0.65)	1.29 (0.9)	1.11 (0.43)	1.17 (0.48)	1.13 (0.84)	0.88 (0.56)	0.88 (0.6)	0.97 (0.75)
9 DSM-IV hyperactive-imp. Sx	1.70 (0.72)	1.77 (0.78)	1.53 (1.02)	1.56 (0.79)	1.82 (0.54)	1.34 (1.00)	1.00 (0.61)	1.41 (0.64)	1.13 (0.81)	0.72 (0.55)	0.98 (0.54)	0.50 (0.6)	0.57 (0.57)	0.79 (0.52)	0.63 (0.67)
All 18 DSM-IV symptoms	2.04 (0.55)	1.96 (0.54)	1.84 (0.63)	1.84 (0.56)	1.93 (0.49)	1.62 (0.73)	1.24 (0.5)	1.52 (0.52)	1.21 (0.75)	0.91 (0.43)	1.08 (0.45)	0.81 (0.58)	0.72 (0.52)	0.83 (0.47)	0.80 (0.59)
CDI															
CDI by parent	21.58 (4.84)	22.4 (6.11)	18.38 (2.2)	19.83 (4.02)	19.00 (4.38)	16.5 (3.74)	18.09 (3.29)	17.89 (4.57)	16.13 (3.72)	17.17 (2.62)	18.06 (4.65)	14.88 (2.8)	16.77 (2.93)	16.63 (3.29)	16.14 (5.76)
CDI by child	25.75 (3.57)	25.35 (3.8)	28.00 (6.68)	26.71 (3.57)	26.26 (3.62)	30.00 (6.87)	25.13 (2.2)	27.11 (4.16)	30.63 (8.94)	24.30 (4.72)	26.72 (3.36)	28.63 (7.58)	26.55 (4.5)	27.00 (3.54)	29.29 (7.39)

[a]At week 21, "Placebo" means 13 weeks of placebo and 8 weeks of zinc compared with 21 weeks of zinc for the others.

Zinc_1 = 15 mg zinc in the morning; Zinc_2 = 15 mg zinc two times per day.

CDI = children's depression inventory; AHDH = attention-deficit/hyperactivity disorder; DMS-IV = *Diagnostic and Statistical Manual fourth edition*; ODD = oppositional defiant disorder; SNAP = Swanson, Nolan, and Pelham rating; Sx = symptoms.

Table 6B. Means (Standard Deviation) of Clinical Measures: Teacher's Rating

	Baseline		*Week 8*		*Week 10*		*Week 13*		*Week 21*[a]	
Mean (SD)/time	*Placebo*	*Zinc*	*Placebo*	*Zinc*	*Placebo*	*Zinc*	*Placebo*	*Zinc*	*Placebo*	*Zinc*
SNAP 9 DSM-IV inattentive Sx	2.35 (0.51)	2.26 (0.57)	2.03 (0.79)	2.16 (0.73)	1.64 (0.66)	1.61 (0.75)	1.07 (0.59)	1.20 (0.57)	1.10 (0.55)	1.51 (0.65)
SNAP 9 DSM-IV hyperactive-imp. Sx	1.78 (0.89)	1.78 (0.7)	1.57 (1.02)	1.73 (0.75)	1.36 (0.89)	1.2 (0.7)	0.66 (0.80)	0.93 (0.62)	0.78 (0.74)	1.04 (0.48)
SNAP All 18 DSM-IV ADHD Sx	2.07 (0.59)	2.02 (0.41)	1.80 (0.78)	1.95 (0.52)	1.50 (0.69)	1.41 (0.6)	0.87 (0.58)	1.07 (0.53)	0.94 (0.59)	1.27 (0.47)
SNAP DSM-IV ODD symptoms	0.71 (0.76)	1.27 (0.94)	0.7 (0.71)	1.2 (0.98)	0.51 (0.61)	0.76 (0.75)	0.32 (0.40)	0.85 (0.67)	0.44 (0.45)	0.71 (0.64)
Conners Teacher Rating Total and Factors										
Overall	82.82 (24.89)	91.65 (23.05)	74.64 (29.50)	85.41 (28.48)	57.5 (29.41)	60.42 (27.77)	31.83 (23.46)	53.63 (29.44)	40.45 (30.94)	57.57 (25.35)
Global index	0.88 (0.38)	1.05 (0.4)	0.78 (0.36)	0.99 (0.47)	0.63 (0.39)	0.83 (0.46)	0.43 (0.50)	0.83 (0.64)	0.45 (0.42)	0.80 (0.49)
Oppositional	1.77 (0.50)	1.81 (0.64)	1.67 (0.66)	1.68 (0.75)	1.18 (0.73)	0.98 (0.64)	0.67 (0.49)	0.85 (0.67)	0.79 (0.64)	0.98 (0.53)
Cognitive problems	1.82 (0.64)	1.95 (0.48)	1.67 (0.85)	1.88 (0.63)	1.37 (0.79)	1.31 (0.56)	0.72 (0.58)	0.98 (0.59)	0.80 (0.71)	1.19 (0.60)
Hyperactive	1.74 (0.65)	1.84 (0.55)	1.49 (0.7)	1.8 (0.73)	1.26 (0.76)	1.23 (0.55)	0.63 (0.40)	0.89 (0.64)	1.03 (0.64)	1.09 (0.64)
Anxious shy	1.13 (0.43)	1.19 (0.47)	0.95 (0.56)	1.02 (0.49)	0.76 (0.52)	0.79 (0.44)	0.35 (0.47)	0.91 (0.59)	0.53 (0.53)	0.85 (0.39)
Perfectionistism	0.25 (0.48)	0.42 (0.56)	0.32 (0.7)	0.39 (0.58)	0.05 (0.15)	0.37 (0.47)	0.25 (0.58)	0.44 (0.44)	0.27 (0.34)	0.29 (0.32)
Social problems	1.23 (0.65)	1.29 (0.55)	1.02 (0.52)	1.32 (0.65)	0.68 (0.57)	0.92 (0.58)	0.50 (0.37)	0.94 (0.48)	0.64 (0.60)	1.04 (0.50)
ADHD index	1.76 (0.50)	1.75 (0.41)	1.60 (0.65)	1.68 (0.46)	1.27 (0.66)	1.24 (0.59)	0.67 (0.56)	0.99 (0.42)	0.82 (0.79)	1.04 (0.43)
Restless/impulsive	0.39 (0.51)	0.62 (0.55)	0.3 (0.43)	0.68 (0.73)	0.18 (0.34)	0.61 (0.57)	0.21 (0.45)	0.56 (0.66)	0.20 (0.35)	0.46 (0.66)
Emotional lability	1.36 (0.47)	1.48 (0.44)	1.27 (0.43)	1.30 (0.43)	1.08 (0.63)	1.05 (0.5)	0.63 (0.64)	1.09 (0.69)	0.70 (0.59)	1.14 (0.5)
9 DSM IV inattentative Sx	1.90 (0.84)	2.03 (0.54)	1.83 (0.92)	2.01 (0.73)	1.47 (0.90)	1.22 (0.7)	0.62 (0.78)	0.94 (0.75)	0.83 (0.76)	1.30 (0.56)
9 DSM IV hyperactive-imp. Sx	1.86 (0.63)	1.85 (0.54)	1.61 (0.72)	1.81 (0.7)	1.34 (0.78)	1.24 (0.53)	0.64 (0.45)	0.86 (0.61)	1.06 (0.68)	1.06 (0.59)
All 18 DSM IV symptoms	1.88 (0.69)	1.93 (0.5)	1.71 (0.79)	1.90 (0.67)	1.40 (0.81)	1.23 (0.58)	0.63 (0.58)	0.90 (0.65)	0.94 (0.69)	1.17 (0.53)

[a]At week 21, "Placebo" means 13 weeks of placebo and 8 weeks of zinc compared with 21 weeks of zinc for the others.

Because the interim analysis suggested that the morning dose of zinc was responsible for effects rated by teachers and we did not expect an effect on teacher ratings from the afternoon dose, and because so many teacher ratings were missing because of vacations, the two zinc dosing schedules (morning only and two times per day) were collapsed for teacher ratings.

AHDH = attention-deficit/hyperactivity disorder; DMS-IV = *Diagnostic and Statistical Manual fourth edition*; ODD = oppositional defiant disorder; SNAP = Swanson, Nolan, and Pelham rating; Sx = symptoms.

Table 7. Laboratory Cognitive-Motor Tests

	Baseline			*Week 8*			*Week 10*			*Week 13*			*Week 21*[a]		
Measure	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*
Short term recognition memory (MMF)															
Accuracy	81.42 (10.28)	76.46 (14.76)	73.44 (11.34)	104.17 (120.9)	76.97 (11.98)	78.12 (7.64)	84.66 (9.04)	78.01 (12.93)	78.65 (10.55)	82.95 (10.89)	81.13 (11.7)	84.90 (9.69)	85.96 (7.76)	82.41 (9.54)	85.18 (4.11)
Response time	1.88 (0.47)	1.93 (0.76)	1.99 (0.37)	2.23 (1.85)	1.95 (0.8)	2.08 (0.31)	1.77 (0.74)	1.65 (0.53)	2.32 (0.74)	1.76 (0.63)	1.59 (0.44)	1.80 (0.55)	1.64 (0.48)	1.58 (0.63)	1.85 (0.36)
Response speed	0.65 (0.16)	0.65 (0.16)	0.58 (0.14)	0.67 (0.17)	0.71 (0.17)	0.59 (0.06)	0.75 (0.25)	0.77 (0.17)	0.57 (0.15)	0.78 (0.28)	0.81 (0.26)	0.73 (0.23)	0.81 (0.3)	0.84 (0.27)	0.63 (0.14)
CPT:															
pers_value_p	14.00 (21.31)	11.4 (17.92)	19.25 (26.36)	19.75 (29.92)	13.44 (17.97)	28.63 (27.66)	13.95 (22.57)	8.44 (13.66)	27.57 (29.69)	12.09 (20.06)	10.88 (13.7)	23.13 (28.49)	16.05 (21.64)	9.26 (11.39)	28.86 (34.96)
pers_tscore_p	55.42 (17.4)	52.69 (18.64)	61.26 (30.55)	60.54 (25.4)	54.81 (17.74)	71.68 (31.89)	55.26 (17.03)	50.92 (14.31)	72.19 (34.22)	54.54 (18.29)	53.26 (14.26)	67.48 (33)	57.97 (20.11)	51.51 (10.74)	74.54 (40.37)
rt_blk_chng_val_v	0.00 (0.04)	0.03 (0.06)	0.04 (0.05)	0.00 (0.04)	0.01 (0.03)	0.01 (0.04)	0.00 (0.02)	0.01 (0.05)	0.01 (0.04)	0.02 (0.05)	0.02 (0.04)	0.01 (0.04)	0.03 (0.04)	0.01 (0.02)	0.01 (0.04)
rt_blk_chng_tscr_v	49.21 (9.78)	49.62 (13.36)	55.32 (9.37)	48.14 (8.33)	47.96 (9.06)	46.59 (6.75)	47.34 (7.25)	49.01 (12.73)	49.76 (6.95)	51.29 (13.55)	49.1 (10.66)	50.14 (7.52)	52.82 (11.73)	46.62 (8.26)	51.58 (12.59)
se_blk_chng_val_v	0.06 (0.09)	0.09 (0.1)	0.11 (0.08)	0.03 (0.08)	0.02 (0.1)	0.03 (0.13)	−0.01 (0.09)	0.03 (0.13)	0.06 (0.11)	0.03 (0.12)	0.02 (0.09)	0.05 (0.14)	0.08 (0.13)	0.00 (0.09)	0.07 (0.08)
se_blk_chng_tscr_v	47.78 (9.01)	48.91 (11)	52.22 (6.88)	47.06 (6.25)	44.31 (9.01)	45.39 (11.12)	43.07 (6.43)	44.94 (11.24)	48.13 (8.72)	45.65 (10.31)	45.25 (8.57)	47.00 (11.4)	49.88 (10.77)	42.64 (7.13)	47.07 (7.33)
Rt_isi_chng_val_i	0.09 (0.06)	0.15 (0.21)	0.10 (0.05)	0.13 (0.08)	0.14 (0.06)	0.10 (0.05)	0.20 (0.42)	0.13 (0.1)	0.10 (0.06)	0.10 (0.08)	0.12 (0.1)	0.09 (0.04)	0.08 (0.08)	0.14 (0.1)	0.09 (0.09)
Rt_isi_chng_tscr_i	50.87 (10.78)	55.99 (15.22)	53.00 (10.43)	58.12 (14.46)	58.89 (12.19)	53.85 (11.13)	56.36 (14.05)	61.35 (20.49)	57.24 (11.91)	52.47 (10.71)	58.39 (18.06)	51.99 (5.41)	51.73 (12.02)	59.8 (17.43)	52.45 (11.87)
Se_isi_chng_val_i	0.08 (0.19)	0.21 (0.23)	0.13 (0.19)	0.15 (0.17)	0.17 (0.15)	0.20 (0.19)	0.41 (1.3)	0.18 (0.23)	0.24 (0.15)	0.12 (0.22)	0.14 (0.22)	0.13 (0.1)	0.14 (0.21)	0.22 (0.22)	0.07 (0.15)
se_isi_chng_tscr_i	48.56 (9.97)	54.47 (11.55)	51.38 (11.71)	52.34 (10.02)	53.84 (8.69)	54.92 (9.78)	52.95 (11.74)	54.41 (13.37)	56.71 (8.53)	50.97 (12.76)	51.98 (12.72)	50.06 (6.23)	51.70 (11.5)	56.28 (13.55)	48.82 (8.25)
hit_rt_err_tscr	52.25 (10.9)	56.44 (11.87)	55.01 (10.3)	55.66 (13.81)	56.74 (9.36)	57.71 (7.94)	51.93 (13.06)	58.87 (14.16)	55.42 (8.92)	51.64 (10.94)	56.91 (11.73)	54.37 (8.41)	50.67 (13.66)	56.34 (13.24)	54.00 (10.01)
hit_rt_err_val	13.19 (9.37)	15.08 (8.94)	15.48 (8.93)	17.00 (14.81)	15.41 (7.64)	17.05 (7.05)	13.52 (10.87)	19.66 (17.47)	15.27 (8.16)	12.47 (8.16)	16.59 (11.09)	14.53 (7.00)	12.65 (10.07)	16.76 (12.5)	14.67 (7.83)
hit_rt_tscr	51.50 (14.07)	61.17 (13.72)	54.76 (10.75)	56.15 (16.45)	58.94 (7.09)	54.77 (10.17)	52.93 (14.45)	61.46 (9.2)	53.06 (16.86)	51.44 (14.03)	60.08 (10.3)	55.56 (18.19)	52.07 (14.06)	60.20 (12.00)	56.47 (12.35)
hit_rt_val	441.83 (120.8)	500.67 (104.09)	475.43 (94.49)	477.17 (154.59)	482.32 (72.31)	474.94 (81.67)	445.02 (122.59)	513.32 (104.81)	465.87 (121.9)	434.27 (112.52)	505.61 (120.78)	491.06 (137.85)	433.09 (116.25)	530.28 (171.47)	493.59 (88.59)
omission_val	21.75 (33.87)	20.8 (24.73)	15.75 (11.89)	28.13 (42.76)	27.67 (28.81)	14.88 (10.13)	23.29 (40.18)	36.5 (63.44)	14.14 (13.31)	15 (20)	21.71 (38.17)	16.63 (14.77)	17.20 (29.00)	22.47 (38.93)	10.14 (9.82)
omission_ts	54.77 (22.3)	52.52 (14.83)	49.37 (7.46)	59.33 (28.14)	59.39 (19.04)	48.71 (6.18)	56.10 (27.3)	65.22 (44.56)	48.53 (8.82)	50.17 (12.18)	54.16 (24.21)	49.98 (9.31)	52.20 (18.7)	54.28 (23.05)	45.46 (5.96)
commission_val	23.79 (8.47)	20.15 (7.86)	27.13 (5.41)	21.46 (8.76)	19.44 (7.23)	26.25 (8.28)	21.00 (9.93)	17.39 (9.42)	24.29 (12.05)	21.14 (9.61)	18.18 (9.69)	22.5 (11.82)	20.9 (9.72)	17.00 (9.17)	21.14 (12.6)
commission_ts	49.79 (11.1)	42.40 (14.89)	53.06 (9.03)	46.25 (11.79)	42.66 (10.56)	51.58 (13.92)	45.48 (13.96)	39.76 (13.48)	48.58 (19.75)	45.72 (13.26)	40.92 (14.22)	45.92 (17.95)	45.25 (14.12)	39.40 (13.3)	43.60 (19.07)
resp_style	0.79 (0.64)	0.81 (0.55)	0.94 (0.8)	0.93 (0.61)	0.81 (0.41)	0.83 (0.51)	0.93 (0.91)	1.16 (1.28)	0.83 (0.53)	0.92 (1.04)	1.06 (0.97)	1.49 (1.86)	0.77 (0.7)	1.04 (1.06)	1.36 (1.75)
resp_tscore	51.18 (12.44)	51.58 (9.85)	53.71 (16.91)	55.62 (14.4)	52.82 (8.28)	51.67 (10.99)	54.06 (17.3)	57.75 (18.69)	51.87 (11.66)	53.27 (16.86)	54.14 (12.13)	58.6 (20.67)	51.73 (14.57)	55.67 (17.51)	56.54 (20.55)
var_tscr	51.07 (9.39)	54.27 (11.76)	54.90 (9.12)	53.72 (11.25)	54.10 (10.58)	55.94 (7.36)	50.56 (12.44)	55.64 (13.15)	54.16 (9.45)	51.49 (11.11)	54.25 (11.28)	51.20 (9.26)	50.86 (11.82)	54.69 (12.23)	50.93 (9.83)
var_val	25.10 (20.78)	27.19 (20.76)	33.1 (23.47)	33.64 (32.16)	31.93 (25.06)	34.09 (18.87)	28.82 (31.57)	42.93 (51.45)	31.40 (20.76)	28.06 (27.63)	32.91 (27.01)	25.9 (21.56)	27.00 (25.23)	37.16 (37.69)	25.94 (20.7)
detect_tscr	49.11 (10.41)	47.14 (15.56)	54.72 (6.95)	49.71 (13.38)	45.19 (11.26)	52.52 (13.06)	45.50 (14.57)	46.57 (17.77)	43.13 (19.91)	46.88 (17.97)	42.34 (12.17)	41.51 (17.19)	43.55 (19.4)	40.32 (12.2)	43.66 (20.76)
detect_val	0.39 (0.34)	0.37 (0.37)	0.16 (0.21)	0.35 (0.45)	0.46 (0.36)	0.20 (0.37)	0.47 (0.44)	0.42 (0.56)	0.49 (0.54)	0.43 (0.54)	0.54 (0.35)	0.55 (0.55)	0.52 (0.61)	0.63 (0.4)	0.47 (0.66)
Total_seat_movement	0.48 (0.49)	0.49 (0.34)	0.74 (0.95)	0.35 (0.4)	0.45 (0.45)	0.49 (0.52)	0.55 (0.58)	0.55 (0.57)	0.32 (0.21)	0.46 (0.47)	0.28 (0.23)	0.19 (0.17)	0.37 (0.32)	0.34 (0.18)	0.27 (0.3)

[a]At week 21, "Placebo" means 13 weeks of placebo and 8 weeks of zinc compared with 21 weeks of zinc for the others.
MMF = Memory for Matching Figures; CPT = Continuous Performance Test.

Table 8. Clinical Outcomes Effect Sizes

		Mean change from baseline			*Effect size (Cohen's* d)	
	Week	*Placebo,* n = *24*	*Zn AM,* n = *20*	*Zn b.i.d.,* n = *8*	*Zn AM (15 mg)*	*Zinc_b.i.d. (30 mg)*
Teacher-rated 9 inattentive symptoms	8	−0.45 (0.94)	−0.14 (0.88)		0.34	
	10	−0.87 (0.70)	−0.70 (0.87)		0.22	
	13	−1.39 (0.78)	−1.05 (0.80)		0.43	
Parent-rated 9 inattentive symptoms	8	−0.25 (0.45)	−0.40 (0.56)	−0.32 (0.40)	−0.30	−0.17
	10	−0.71 (0.61)	−0.68 (0.70)	−0.76 (0.44)	0.05	−0.09
	13	−1.16 (0.64)	−1.06 (0.61)	−1.19 (0.27)	0.16	−0.05
Teacher-rated all 18 ADHD symptoms	8	−0.47 (0.87)	−0.28 (0.81)		0.23	
	10	−0.81 (0.64)	−0.82 (0.84)		−0.01	
	13	−1.44 (0.66)	−1.19 (0.76)		0.35	
Parent-rated all 18 ADHD symptoms	8	−0.26 (0.41)	−0.34 (0.53)	−0.31 (0.24)	−0.17	−0.14
	10	−0.71 (0.50)	−0.69 (0.66)	−0.51 (0.30)	0.04	0.44
	13	−1.17 (0.54)	−1.06 (0.71)	−1.10 (0.44)	0.18	0.14
Optimal mg AMPH dose	13	13.32 (6.30)	13.55 (6.93)	7.5 (4.43)	0.04	−1.09
Optimal mg/kg AMPH dose	13	0.35 (0.12)	0.35 (0.14)	0.22 (0.13)	0.00	−1.04

Summary of primary outcome effect sizes (Cohen's *d*). Negative *d* favors zinc.
Phase 1 (8 weeks, inattentive Sx): teacher $d = 0.34$; parent AM dose $d = -0.31$; b.i.d. dose $d = -0.14$.
Phase 2 (10 weeks, all 18 ADHD Sx): teacher $d = -0.01$; parent AM dose $d = 0.04$; b.i.d. dose $d = 0.44$.
Phase 3 (13-week AMPH dose): with AM dose zinc $d = 0.04$ and 0.00; with b.i.d. dose zinc $d = -1.09$ and -1.04.
Lower score better. Negative ES favors zinc. Zinc in AM only = 15 mg elemental zinc per day; zinc b.i.d. = 30 mg elemental zinc per day. Due to the end of school year, teacher ratings with b.i.d, dosing were not complete enough to analyze separately, and no difference between qAM and b.i.d. dosing was expected on teacher ratings, so all zinc teacher ratings were collapsed for analysis.
AMPH = d-amphetamine; AM = morning; Zn = zinc; b.i.d. = two times per day; ADHD = attention deficit/hyperactivity disorder.

were also inconsistent, although a bit more favorable to zinc, especially b.i.d..

Effect sizes

The planned outcomes were effect sizes (Cohen's d, difference from placebo in change score divided by pooled SD of change scores), shown in Tables 8 and 9 for key outcomes. For phase 1 the planned primary outcome was inattentive symptoms rated by parents and teachers; parent ratings showed a small effect favoring zinc (−0.31 for single-dose, −0.14 for b.i.d. dosing), but teacher ratings showed an opposite tendency (0.34). For phase 2, outcome was all 18 ADHD symptoms rated by parent and teacher; parent ratings favored placebo (0.04–0.44) although teacher ratings nominally/negligibly favored zinc ($d = -0.01$). In sum, the primary clinical outcomes (parent- and teacher-rated ADHD symptoms) showed no consistent tendency of superiority of zinc over placebo. The phase 3 primary outcome was week 13 optimized dose of amphetamine; this did show the predicted >20% lower dose of amphetamine (actually >43% lower for absolute mg doses, >37% lower for mg/kg doses) for b.i.d. zinc, but not for single-dose zinc, for which there was no difference in doses.

Physiological checks (Table 10) and AEs (Tables 11A–11C) were generally unremarkable. Most differences between placebo and zinc supplementation were not clinically significant. Before addition of amphetamine, the placebo group had nominally more AEs per participant (3.96) than either single-dose (3.45) or b.i.d. (3.38) zinc. Counting only those the clinician thought were related

Table 9. Neuropsychological Lab Effect Sizes

		Assess	*Mean change from baseline*			*Effect size (ES)*	
Measures	*Variables*	*Week*	*Placebo,* n = *24*	*Zn AM,* n = *20*	*Zn b.i.d.,* n = *8*	*Zn AM (15 mg)*	*Zn b.i.d. (30 mg)*
CPT	Omission errors ↓	8	6.38 (24.46)	11.17 (19.59)	−0.88 (8.29)	0.21	−0.36
		10	3.67 (38.78)	14.94 (62.54)	2.00 (12.99)	0.22	−0.05
		13	−4.41 (26.97)	−0.59 (21.10)	0.86 (7.79)	0.16	0.24
	Commission errors ↓	8	−2.33 (5.32)	−0.72 (5.56)	−0.88 (4.58)	0.30	0.28
		10	−2.05 (5.70)	−3.22 (8.66)	−3.29 (7.91)	−0.17	−0.20
		13	−2.27 (5.25)	−3.24 (5.77)	−4.63 (8.18)	−0.18	−0.39
MMF test	Accuracy ↑	8	−2.26 (8.60)	0.22 (11.66)	4.69 (13.44)	0.25	0.71
		10	1.52 (9.05)	1.85 (11.36)	5.21 (7.00)	0.03	0.43
		13	−0.20 (11.81)	5.39 (11.00)	11.46 (6.95)	0.49	1.10
	Seat movement ↓	8	−0.13 (0.54)	−0.03 (0.53)	−0.26 (0.55)	0.19	−0.24
		10	0.13 (0.66)	0.04 (0.58)	−0.42 (0.95)	−0.15	−0.75
		13	0.05 (0.54)	−0.23 (0.38)	−0.55 (0.89)	−0.60	−0.95

Polarity indicated by ↑ for higher score better (positive ES favors zinc) and ↓ for lower score better (neg. ES favors zinc).
Zn = zinc; AM = morning; b.i.d. = two times per day; CPT = Continuous Performance Test; MMF = Memory for Matching Figures.

TABLE 10. PHYSIOLOGICAL MEASURES

	Baseline			Week 8			Week 10			Week 13			Week 21[a]		
Mean (SD)/time	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*
Weight	36.7 (12.1)	36.8 (13.8)	32.4 (8.5)	37.6 (12.3)	39.2 (14.5)	33.2 (8.9)	37.4 (11.8)	38.4 (14.3)	32.8 (8.7)	37.4 (11.7)	37.4 (14.6)	32.5 (8.5)	37.3(11.8)	37.0 (12.4)	30.0 (7.0)
Heart rate	83.5 (15.0)	82.5 (12.8)	85.5 (8.7)	83.1 (12.1)	84.21 (8.2)	85.0 (7.6)	82.6 (12.4)	84.1 (10.6)	83.3 (6.8)	90.0 (13.8)	86.4 (13.6)	87.3 (8.9)	90.9 (13.6)	82.6 (8.6)	88.6 (8.8)
Systolic pressure	104.7 (9.4)	105.4 (13.8)	102.0 (7.8)	105.5 (9.3)	104.6 (12.4)	100.2 (11.2)	106.7 (8.7)	108.2 (13.9)	106.5 (10.0)	102.8 (10.7)	103.1 (14.3)	102.9 (8.2)	99.8 (12.0)	105.2 (10.4)	96.9 (8.8)
Diastolic pressure	67.8 (8.0)	67.5 (10.0)	66.5 (7.5)	68.2 (7.8)	69.9 (11.2)	65.3 (6.2)	71.1 (8.4)	69.8 (13.0)	70.0 (6.0)	65.7 (7.4)	66.8 (9.2)	67.0 (8.2)	62.9 (7.4)	68.9 (8.7)	62.0 (8.3)

[a]At week 21, "Placebo" means 13 weeks of placebo and 8 weeks of zinc compared with 21 weeks of zinc for the others. All also received amphetamine after week 8.

to the study treatment, 34 occurred in the 24 placebo participants, 24 in the 20 single-dose zinc participants, and 4 in the 8 b.i.d. zinc participants. Most AEs were mild or moderate. Of those rated severe, 20 occurred with placebo, 24 with single-dose zinc, and 5 with b.i.d. zinc. Addition of amphetamine, as expected, increased the AEs for all participants, with comparable distribution across treatment condition. Affective blunting from amphetamine occurred in 21% of the placebo group, but in only 11% of the zinc-supplemented participants, about a 2:1 ratio, but other amphetamine side effects occurred slightly more in the b.i.d. zinc group. Overall rate and severity of AEs showed no trend of difference between zinc and placebo.

Dietary intake on the Kids' Food Questionnaire (Table 12) generally showed adequate mean intake of various nutrients, but several, including zinc, were at borderline minimal intake. Changes over time were negligible except for a general tendency for the b.i.d. zinc group to eat slightly less of everything at week 8. These data will be analyzed in more detail separately.

Moderator/mediator analyses

For the moderator (and mediator) analyses, the three measures of zinc level were changed to *z*-scores based on the sample mean and SD for each measure. Then, the three *z*-scores for each participant were averaged. Thus, the sample was split into those with *z*-scores below (<0) and above (>0) zero, reflecting relative zinc status. For mediator analyses, the *z*-scores based on baseline mean and SD were used to derive change scores of zinc level from baseline to week 8 and these were correlated with symptom change scores at weeks 8, 10, and 13. As expected, no moderator or mediator analyses were significant ($p = 0.7$–0.8 for parent ratings, 0.24–0.34 for teacher ratings), and in general were not instructive, but the parent-rated ADHD symptom moderator graph (Fig. 2) suggests a tendency for nominal zinc superiority over placebo in those with low but not high baseline zinc. Because Bilici et al. (2004) reported a greater effect in older children, we also ran a moderator analysis with a median split on age; it showed essentially the same results regardless of age.

Discussion

Only one of the three main hypotheses was upheld, a significantly lower optimal dose of amphetamine for those with zinc supplement, whether based on absolute mg/day (43% lower) or on the weight-adjusted mg/kg per day (37% lower). Whether this lower dose was responsible for halving the rate of affective blunting with amphetamine (from 21% to 11%) we cannot tell from the data available, but other amphetamine side effects did not show a similar trend. If upheld by further study, the possibility of a lower optimal dose of stimulant may have some clinical value.

An important issue regarding this possible zinc–drug interaction may be prior medication history. Possibly prior drug exposure could have some sort of priming or inhibiting effect, or prior failure on stimulant might predispose to seeking alternative treatment such as zinc. In this case, only 12 of the 52 participants had previous exposure to amphetamine and only 23 had prior exposure to any ADHD medication at all, with atomoxetine being the most common prior drug (with some participants trying more than one, 14 took atomoxetine and 11 methylphenidate). More to the point, only 5 took any ADHD drug within 30 days of study entry: Two in placebo stopped methylphenidate patch (30 mg), two in zinc stopped amphetamine (20 and 25 mg), and one in zinc stopped Concerta (18 mg). Because most families who had tried ADHD drugs had apparently given up on them well before study entry, we might

Table 11A. Numbers of Adverse Effects in Each Treatment Group Before/After Week 8

	Through week 8: Zinc monotherapy			*After Week 8: with amphetamine*		
	Group			*Group*		
Adverse effects	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*
Affective blunting	1 (4.17%)	1 (5%)	0 (0%)	6 (25%)	4 (20%)	0 (0%)
Anxiety	6 (25%)	6 (30%)	2 (25%)	5 (20.83%)	9 (45%)	3 (37.5%)
Appetite	4 (16.7%)	3 (15%)	4 (50%)	17 (70.8%)	15 (75%)	8 (100%)
Cardiovascular	0 (0%)	0 (0%)	0 (0%)	2 (8.33%)	0 (0%)	0 (0%)
Central nervous system reactions	0 (0%)	0 (0%)	1 (12.5%)	7 (29.17%)	1 (5%)	0 (0%)
Dental	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (10%)	1 (12.5%)
Depression	5 (20.8%)	7 (35%)	2 (25%)	9 (37.5%)	11 (55%)	4 (50%)
Fatigue	6 (25%)	2 (10%)	2 (25%)	2 (8.33%)	5 (25%)	5 (62.5%)
Fever	0 (0%)	2 (10%)	1 (12.5%)	1 (4.17%)	2 (10%)	1 (12.5%)
Harm to self and others	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Head, eyes, ears, nose, and throat	3 (12.5%)	3 (15%)	1 (12.5%)	2 (8.33%)	4 (20%)	1 (12.5%)
Headache	13 (54.2%)	5 (25%)	2 (25%)	8 (33.3%)	8 (40%)	3 (37.5%)
Hypersensitivity reaction	1 (4.17%)	3 (15%)	0 (0%)	5 (20.8%)	0 (0%)	1 (12.5%)
Irritability	10 (41.7%)	9 (45%)	5 (62.5%)	14 (58.3%)	9 (45%)	6 (75%)
Musculoskeletal	4 (16.7%)	1 (5%)	0 (0%)	2 (8.33%)	1 (5%)	0 (0%)
Other	2 (8.33%)	0 (0%)	0 (0%)	2 (8.33%)	0 (0%)	0 (0%)
Respiratory	5 (20.8%)	8 (40%)	0 (0%)	4 (16.7%)	5 (25%)	0 (0%)
Slapped in Face	1 (4.17%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
School performance	0 (0%)	1 (5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Skin	3 (12.5%)	1 (5%)	1 (12.5%)	2 (8.33%)	2 (10%)	2 (25%)
Sleep	4 (16.7%)	0 (0%)	1 (12.5%)	16 (66.7%)	8 (40%)	6 (75%)
Stereotypical behaviors	5 (20.8%)	3 (15%)	1 (12.5%)	9 (37.5%)	7 (35%)	2 (25%)
Stomach aches	13 (54.2%)	6 (30%)	3 (37.5%)	11 (45.8%)	7 (35%)	3 (37.5%)
Other gastrointestinal	5 (20.8%)	5 (25%)	1 (12.5%)	3 (12.5%)	4 (20%)	0 (0%)
URI	4 (16.7%)	2 (10%)	0 (0%)	2 (8.33%)	1 (5%)	0 (0%)
Total number of AEs	95	69	27	129	106	46
Number of AEs/participant	3.96	3.45	3.38	5.38	5.3	5.75

AEs = adverse events; URI = upper respiratory infection.

assume considerable dissatisfaction with the results, so there could have been some self-selection for poor responders. Nevertheless, the mean response to amphetamine during the study was good whether on placebo or zinc. In view of the low proportion with prior amphetamine and even lower with recent medication, we do not think this was a significant factor in the optimal-dosage difference.

Regarding the two failed hypotheses

Lack of statistical significance was not an issue because the study was not powered for statistical significance, only for exploration of clinical effect sizes. Hypotheses were stated in terms of effect sizes and graphed curves. However, even these did not come out as predicted based on previous data and the mideastern trials. Although both treatment groups showed the expected dramatic improvement with optimized amphetamine, differences between zinc and placebo were not impressive. Most of the primary outcomes failed to reach the hypothesized effect sizes in the predicted direction. An exception was parent-rated inattention for 15 mg zinc monotherapy ($d = -0.31$). This was balanced by similar effect sizes in the opposite direction on other ratings, resulting in a failed trial for those two hypotheses based on the primary measures.

In mild contrast to the equivocal primary measures and secondary clinical measures, the secondary objective laboratory neuropsychological measures showed a pattern a bit more favorable to zinc, especially at the 30 mg/day dose. For example, accuracy on the Matching-to-Familiar Figures Test yielded medium ($d = 0.43$) to large ($d = 1.10$) effect sizes favoring 30 mg zinc over placebo. Further, except for omission errors, the neuropsychological tests suggest greater advantage of zinc over placebo at the 13-week optimized amphetamine dose than at 8 weeks with monotherapy. If upheld by replication, this might be either interaction of zinc with stimulant or simply duration of zinc supplementation.

The significant increase in urine zinc compared with placebo, coupled with the failure of three different blood tests to show more than a hint of rise in zinc, suggested that administered zinc is at least partially absorbed but immediately excreted. However, an all-day pharmacokinetic study of five ADHD patients with 15 mg zinc in the AM failed to demonstrate any appreciable difference from two non-ADHD normal control children in shape of curve or area under the curve.

The failure to find an effect on symptom severity is not unique. In a 6-month Guatemalan trial published while this report was in press, DiGirolamo et al. (2010) randomized 674 children in grades 1–4 not selected for ADHD diagnosis to 10 mg/day zinc as the oxide or 10 mg/day glucose for 5 days a week. Both groups improved significantly, but the difference between groups was not significant for either internalizing or externalizing symptoms, including hyperactivity. However, increases in serum zinc correlated significantly with improvement in internalizing symptoms and social skills.

It is difficult to know how to interpret failed trials because of the danger of false-negatives. There are many possible reasons for a failed trial. The most obvious and most tempting explanation is that

Table 11B. Adverse Event Severity by Groups Before/After Week 8

		Through week 8: Zinc monotherapy			*After Week 8: with amphetamine*		
Category	*Severity*	*Placebo*	*Zinc_1*	*Zinc_2*	*Placebo*	*Zinc_1*	*Zinc_2*
Affective blunting	1	1 (4.17%)	1 (5%)	0 (0%)	6 (25%)	3 (15%)	0 (0%)
	2	1 (4.17%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Anxiety	1	5 (20.83%)	3 (15%)	0 (0%)	4 (16.67%)	5 (25%)	3 (37.5%)
	2	1 (4.17%)	3 (15%)	2 (25%)	1 (4.17%)	3 (15%)	0 (0%)
	3	1 (4.17%)	3 (15%)	2 (25%)	0 (0%)	1 (5%)	0 (0%)
Appetite	1	2 (8.33%)	3 (15%)	4 (50%)	13 (54.17%)	8 (40%)	6 (75%)
	2	2 (8.33%)	0 (0%)	0 (0%)	3 (12.5%)	6 (30%)	2 (25%)
	3	2 (8.33%)	0 (0%)	0 (0%)	1 (4.17%)	1 (5%)	0 (0%)
Cardiovascular	1	0 (0%))	0 (0%))	0 (0%))	1 (4.17%)	0 (0%)	0 (0%)
	2	0 (0%))	0 (0%))	0 (0%))	1 (4.17%)	0 (0%)	0 (0%)
Central nervous system reactions	1	0 (0%)	0 (0%)	1 (12.5%)	3 (12.5%)	1 (5%)	0 (0%)
	2	0 (0%)	0 (0%)	1 (12.5%)	3 (12.5%)	0 (0%)	0 (0%)
	3	0 (0%)	0 (0%)	1 (12.5%)	1 (4.17%)	0 (0%)	0 (0%)
Dental	1	0 (0%)	0 (0%)	0 (0%))	0 (0%)	2 (10%)	1 (12.5%)
Depression	1	3 (12.5%)	4 (20%)	1 (12.5%)	5 (20.83%)	6 (30%)	3 (37.5%)
	2	2 (8.33%)	3 (15%)	1 (12.5%)	3 (12.5%)	4 (20%)	1 (12.5%)
	3	2 (8.33%)	3 (15%)	1 (12.5%)	1 (4.17%)	1 (5%)	0 (0%)
Fatigue	1	6 (25%)	1 (5%)	2 (25%)	2 (8.33%)	3 (15%)	5 (62.5%)
	2	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
	3	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Fever	1	0 (0%)	1 (5%)	1 (12.5%)	1 (4.17%)	1 (5%)	1 (12.5%)
	3	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Harm to self and others	3	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Head, eyes, ears, nose, and throat	1	2 (8.33%)	1 (5%)	1 (12.5%)	2 (8.33%)	3 (15%)	1 (12.5%)
	2	1 (4.17%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
	3	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Headache	1	9 (37.5%)	2 (10%)	2 (25%)	6 (25%)	4 (20%)	3 (37.5%)
	2	4 (16.67%)	3 (15%)	0 (0%)	2 (8.33%)	3 (15%)	0 (0%)
	3	4 (16.67%)	3 (15%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Hypersensitivity reaction	1	0 (0%)	2 (10%)	0 (0%)	5 (20.83%)	0 (0%)	1 (12.5%)
	2	1 (4.17%)	1 (5%)	0 (0%)	5 (20.83%)	0 (0%)	1 (12.5%)
Irritability	1	6 (25%)	5 (25%)	5 (62.5%)	7 (29.17%)	1 (5%)	5 (62.5%)
	2	3 (12.5%)	4 (20%)	0 (0%)	6 (25%)	6 (30%)	1 (12.5%)
	3	1 (4.17%)	0 (0%)	0 (0%)	1 (4.17%)	2 (10%)	0 (0%)
Musculoskeletal	1	3 (12.5%)	1 (5%)	0 (0%)	1 (4.17%)	1 (5%)	0 (0%)
	2	1 (4.17%)	0 (0%)	0 (0%)	1 (4.17%)	0 (0%)	0 (0%)
Other	1	1 (4.17%)	0 (0%)	0 (0%)	1 (4.17%)	0 (0%)	0 (0%)
	2	1 (4.17%)	0 (0%)	0 (0%)	1 (4.17%)	0 (0%)	0 (0%)
Other gastrointestinal	1	5 (20.83%)	3 (15%)	1 (12.5%)	2 (8.33%)	4 (20%)	0 (0%)
	2	0 (0%)	2 (10%)	0 (0%)	1 (4.17%)	0 (0%)	0 (0%)
Respiratory	1	5 (20.83%)	8 (40%)	0 (0%)	3 (12.5%)	4 (20%)	0 (0%)
	2	5 (20.83%)	8 (40%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
	3	5 (20.83%)	8 (40%)	0 (0%)	1 (4.17%)	0 (0%)	0 (0%)
Slapped by father in face	3	1 (4.17%)	0 (0%)	0 (0%)	0 (0%))	0 (0%))	0 (0%))
School performance	2	0 (0%)	1 (5%)	0 (0%)	0 (0%))	0 (0%))	0 (0%))
Skin	1	1 (4.17%)	0 (0%)	1 (12.5%)	0 (0%)	1 (5%)	1 (12.5%)
	2	1 (4.17%)	1 (5%)	0 (0%)	2 (8.33%)	1 (5%)	1 (12.5%)
	3	1 (4.17%)	0 (0%)	0 (0%)	2 (8.33%)	1 (5%)	1 (12.5%)
Sleep	1	3 (12.5%)	0 (0%)	1 (12.5%)	5 (20.83%)	3 (15%)	3 (37.5%)
	2	1 (4.17%)	0 (0%)	0 (0%)	10 (41.67%)	3 (15%)	3 (37.5%)
	3	1 (4.17%)	0 (0%)	0 (0%)	1 (4.17%)	2 (10%)	0 (0%)
Stereotypical behaviors	1	3 (12.5%)	2 (10%)	0 (0%)	6 (25%)	4 (20%)	1 (12.5%)
	2	1 (4.17%)	1 (5%)	0 (0%)	3 (12.5%)	3 (15%)	0 (0%)
	3	1 (4.17%)	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)	1 (12.5%)
Stomach aches	1	10 (41.67%)	2 (10%)	2 (25%)	7 (29.17%)	6 (30%)	3 (37.5%)
	2	2 (8.33%)	3 (15%)	1 (12.5%)	4 (16.67%)	0 (0%)	0 (0%)
	3	1 (4.17%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
URI	1	2 (8.33%)	1 (5%)	0 (0%)	1 (4.17%)	1 (5%)	0 (0%)
	2	2 (8.33%)	1 (5%)	0 (0%)	1 (4.17%)	0 (0%)	0 (0%)

1 = mild; 2 = moderate; 3 = severe.
URI = upper respiratory infection.

Table 11C. Adverse Events Relationship to the Treatments

Category	Relationship to study Tx	Through week 8: Zinc monotherapy			After week 8: with amphetamine		
		Placebo	Zinc_1	Zinc_2	Placebo	Zinc_1	Zinc_2
Affective blunting	Related	0 (0%)	1 (5%)	0 (0%)	5 (20.83%)	3 (15%)	0 (0%)
	Unrelated	1 (4.17%)	0 (0%)	0 (0%)	1 (4.17%)	1 (5%)	0 (0%)
Anxiety	Related	2 (8.33%)	4 (20%)	1 (12.5%)	4 (16.67%)	7 (35%)	3 (37.5%)
	Unrelated	4 (16.67%)	2 (10%)	1 (12.5%)	1 (4.17%)	2 (10%)	0 (0%)
Appetite	Related	3 (12.5%)	2 (10%)	1 (12.5%)	17 (70.83%)	13 (65%)	8 (100%)
	Unrelated	1 (4.17%)	1 (5%)	3 (37.5%)	0 (0%)	2 (10%)	0 (0%)
Cardiovascular	Related	0 (0%))	0 (0%))	0 (0%))	2 (8.33%)	0 (0%)	0 (0%)
Central nervous system reactions	Related	0 (0%)	0 (0%)	1 (12.5%)	7 (29.17%)	1 (5%)	0 (0%)
Dental	Unrelated	0 (0%))	0 (0%))	0 (0%))	0 (0%)	2 (10%)	1 (12.5%)
Depression	Related	1 (4.17%)	3 (15%)	1 (12.5%)	7 (29.17%)	9 (45%)	4 (50%)
	Unrelated	4 (16.67%)	4 (20%)	1 (12.5%)	2 (8.33%)	2 (10%)	0 (0%)
Fatigue	Related	2 (8.33%)	1 (5%)	0 (0%)	1 (4.17%)	3 (15%)	4 (50%)
	Unrelated	4 (16.67%)	1 (5%)	2 (25%)	1 (4.17%)	2 (10%)	1 (12.5%)
Fever	Unrelated	0 (0%)	2 (10%)	1 (12.5%)	1 (4.17%)	2 (10%)	1 (12.5%)
Harm to self and others	Unrelated	0 (0%)	1 (5%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
Head, eyes, ears, nose, and throat	Unrelated	3 (12.5%)	3 (15%)	1 (12.5%)	2 (8.33%)	4 (20%)	1 (12.5%)
Headache	Related	6 (25%)	2 (10%)	0 (0%)	1 (4.17%)	5 (25%)	3 (37.5%)
	Unrelated	7 (29.17%)	3 (15%)	2 (25%)	7 (29.17%)	3 (15%)	0 (0%)
Hypersensitivity reaction	Unrelated	1 (4.17%)	3 (15%)	0 (0%)	5 (20.83%)	0 (0%)	1 (12.5%)
Irritability	Related	4 (16.67%)	6 (30%)	0 (0%)	13 (54.17%)	9 (45%)	6 (75%)
	Unrelated	6 (25%)	3 (15%)	5 (62.5%)	1 (4.17%)	0 (0%)	0 (0%)
Musculoskeletal	Unrelated	4 (16.67%)	1 (5%)	0 (0%)	2 (8.33%)	1 (5%)	0 (0%)
Other	Related	2 (8.33%)	0 (0%)	0 (0%)	2 (8.33%)	0 (0%)	0 (0%)
Other gastrointestinal	Related	3 (12.5%)	0 (0%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)
	Unrelated	2 (8.33%)	5 (25%)	1 (12.5%)	3 (12.5%)	3 (15%)	0 (0%)
Respiratory	Unrelated	5 (20.83%)	8 (40%)	0 (0%)	4 (16.67%)	5 (25%)	0 (0%)
Slapped in face	Unrelated	1 (4.17%)	0 (0%)	0 (0%)	0 (0%))	0 (0%))	0 (0%))
Skin	Unrelated	3 (12.5%)	1 (5%)	1 (12.5%)	2 (8.33%)	2 (10%)	2 (25%)
Sleep	Related	1 (4.17%)	0 (0%)	0 (0%)	15 (62.5%)	8 (40%)	6 (75%)
	Unrelated	3 (12.5%)	0 (0%)	1 (12.5%)	1 (4.17%)	0 (0%)	0 (0%)
Stereotypical Behaviors	Related	2 (8.33%)	1 (5%)	0 (0%)	8 (33.33%)	4 (20%)	1 (12.5%)
	Unrelated	3 (12.5%)	2 (10%)	1 (12.5%)	1 (4.17%)	3 (15%)	1 (12.5%)
Stomach aches	Related	8 (33.33%)	4 (20%)	0 (0%)	7 (29.17%)	4 (20%)	3 (37.5%)
	Unrelated	5 (20.83%)	2 (10%)	3 (37.5%)	4 (16.67%)	3 (15%)	0 (0%)
URI	Unrelated	4 (16.67%)	2 (10%)	0 (0%)	2 (8.33%)	1 (5%)	0 (0%)

Related = possibly, probably, or definitely related; unrelated = definitely not related.
URI = upper respiratory infection.

there is no difference, in this case between zinc supplementation and placebo—that is, the treatment is ineffective. However, before embracing that conclusion we should consider other possible explanations, especially in view of the three positive Mideastern reports [Bilici et al. (2004) and Uckardes et al. (2009) in Turkey, and Akhondzadeh et al. (2004) in Iran].

One of the common reasons for a failed trial is insufficient power (insufficient sample size). This is usually suspected when a nonsignificant trend in the predicted direction is detected that appears clinically significant. In this case, of course, the hypotheses focused on such a trend without expecting statistical significance, and it was not detected on most clinical measures (although it was for optimal dose of amphetatmine). Further, most nominal differences in primary clinical measures were even in the opposite direction from what was predicted! Therefore, the failure of hypotheses could not be due to inadequate power.

Another possibility for a failed trial could be the wrong sample or treatment targets. In this case the sample was diagnosed by standardized research procedures by experienced child psychiatrists with the aid of a structured interview and comprehensive data collection from multiple informants (parent, child, and teacher). The primary targets of treatment were the DSM-IV symptoms, which are routinely responsive to established ADHD treatments, and other clinical outcome domains were also assessed with disappointing results. Of course, it might be argued that despite the rigorous categorical and dimensional intake diagnostic process, the sample still differed from usual ADHD patients in higher socioeconomic status, lower co-morbidity, and willingness to receive amphetamine despite a great interest in alternative treatment. However, this argument does not seem intuitively persuasive given the comparable degree of symptom severity and the wide-ranging effectiveness of other treatments.

A final sampling problem may be that most of the sample was not deficient in zinc. If they were not deficient in zinc, one would not expect zinc supplementation to help. This explanation is somewhat supported by a moderator analysis of initial zinc level, which did show a minimally better result by visual inspection of parent ratings for those on the low end of the zinc spectrum. However, that was confined to week 8, before addition of amphetamine, and did not appear impressive enough to explain the lack of finding. Therefore,

Table 12. Dietary Nutrient Intake from Block Kids' Food Questionnaire by Parent Informant

	Baseline			*Week 8*		
	Group			*Group*		
Dietary intake	*Placebo mean (SD)*	*Zinc_1 qAM*	*Zinc_2 b.i.d.*	*Placebo mean (SD)*	*Zinc_1 qAM*	*Zinc_2 b.i.d.*
Alpha carotene, mcg	187.7 (208.9)	179.8 (221.1)	757.1 (1,366.9)	286.6 (492.6)	225 (219.3)	432.3 (649.6)
Beta carotene, mcg	1,097.6 (840.8)	1,085.2 (1,041.2)	2,341.2 (3,518.1)	1,268.1 (1,562.8)	1,086.2 (744.7)	1,387.2 (1,426.3)
Calcium, mg	921 (360.6)	773.5 (353.7)	901.6 (405.6)	923.8 (491.1)	848.2 (282.6)	817.8 (450.4)
Carbohydrate, g	240.4 (81.2)	214.5 (83.5)	241.5 (125.8)	249.6 (132.8)	212.8 (70.2)	173.8 (77.8)
Cholesterol, mg	198.1 (83.3)	207.8 (108.7)	180.5 (93.1)	224.6 (140.2)	219.8 (76.6)	131.4 (50.8)
Copper, mg	1.0 (0.3)	0.9 (0.4)	1 (0.5)	1.1 (0.7)	1.0 (0.3)	0.7 (0.3)
Fat, g	66.6 (22)	59.6 (21.7)	61.9 (25.5)	70 (39)	64.3 (16.7)	47.1 (17.7)
Folate, mcg	350.6 (102.9)	285.9 (124.1)	327 (187.7)	362.2 (202.2)	306 (129.2)	250.4 (110.1)
Iron, mg	13 (4)	10.9 (4.5)	12.2 (5.2)	13.7 (7.1)	11.8 (4.8)	9.6 (3.4)
Linoleic acid, g	12.3 (4)	11.2 (4.6)	10.8 (5.5)	13.2 (8.8)	12.4 (4.2)	8.6 (3.5)
Lycopene, mcg	4,658.6 (2,679.5)	4,673.9 (3,386.2)	5,152.6 (3,078.3)	5,709.4 (3,743.5)	4,417.8 (2,559.9)	3,054 (703.2)
Magnesium, mg	220.4 (67.4)	184.9 (77.9)	204.8 (106)	225.1 (131.7)	197.8 (69.3)	167.8 (72.6)
Oleic acid, g	25.4 (8.8)	22.9 (8.3)	24.3 (10.7)	26.5 (14.7)	24.3 (6.2)	18.1 (6.1)
OMEGA-3, g	1.1 (0.3)	1 (0.4)	0.9 (0.4)	1.2 (0.7)	1.1 (0.4)	0.8 (0.3)
OMEGA-6, g	11 (3.6)	9.9 (4.1)	9.6 (5)	11.8 (8)	11 (3.9)	7.6 (3.3)
Percent fat in diet	33.4% (3.3%)	33.5% (3.3%)	32.5% (4.5%)	33.8% (3.5%)	35.8% (5%)	33% (4%)
Percent carbohydrate	53.2% (4.7%)	53.2% (5%)	54.3% (6%)	53.5% (4.8%)	50.8% (7.1%)	52.8% (4.8%)
Percent protein	14.7% (1.7%)	14.6% (2.2%)	14.3% (2.8%)	14.1% (1.8%)	14.7% (2.3%)	15.5% (2.2%)
Phosphorus, mg	1,184 (372.8)	1,017.2 (425.2)	1,116.1 (449.8)	1,179.1 (635.5)	1,097 (318.2)	960.8 (451.8)
Potassium, mg	2,240.3 (810.1)	1,884.6 (827.9)	2,141.3 (1,118.1)	2,277 (1,431.1)	1,916.3 (577.2)	1,682.8 (662.4)
Protein, g	65.8 (21.3)	57.3 (21.3)	60.5 (23)	65.9 (37)	59.8 (18.5)	49.7 (19)
Riboflavin/B2, mg	2.0 (0.6)	1.6 (0.7)	1.8 (0.7)	2.0 (1.0)	1.8 (0.5)	1.6 (0.8)
Saturated fat, g	23.5 (8.5)	20.7 (7.8)	21.9 (8)	24.6 (13)	22.6 (6.1)	16.9 (8.1)
Selenium, mcg	82.4 (25.7)	74.4 (28.8)	77.7 (28.4)	84.7 (47.3)	79.1 (26.6)	63.5 (26)
Sodium, mg	2,844.5 (961.7)	2,524.4 (942.4)	2,823.5 (1,000.3)	2,917.1 (1,532.4)	2,600.8 (900.9)	2,073.9 (659.6)
Sugar, g	124.7 (45.7)	119.1 (56.4)	125.9 (68.5)	135.1 (76.8)	110.8 (36.3)	91.1 (52.8)
Transfat, g	6.3 (2.6)	5.5 (2.5)	5.6 (4)	6.2 (3.6)	5.6 (2)	4.2 (1.7)
Vitamin A, mcg	551.4 (199.7)	451.2 (257.3)	641.7 (454.3)	560.5 (320.3)	515.5 (201.4)	517 (277)
Vitamin B1, mg	1.5 (0.4)	1.2 (0.5)	1.4 (0.6)	1.5 (0.8)	1.3 (0.4)	1.1 (0.5)
Vitamin B12, mcg	4.7 (1.5)	4 (1.7)	4 (1.1)	4.7 (2.4)	4.4 (1.4)	4 (1.9)
Vitamin B3, mg	18.2 (5.9)	15.5 (5.5)	17 (7.5)	18.4 (9.7)	15.9 (5.5)	13.2 (4.4)
Vitamin C, mg	107.1 (80.8)	98.8 (88.4)	107.2 (92.5)	131.6 (138)	79.8 (52.4)	53.3 (19)
Vitamin D, IU	192.8 (93.4)	154.5 (85.7)	158.4 (76.8)	185 (121.9)	181.8 (62.2)	190 (119.7)
Vitamin E, mg	5.3 (1.7)	4.8 (2.3)	4.8 (3.2)	6.1 (5)	5.6 (2.2)	3.9 (2.1)
Vitamin K, mcg	52.8 (35.7)	53.6 (50.1)	59.6 (76.5)	55.5 (53.3)	44.4 (30.5)	40.5 (22.7)
Vitamin B6, mg	1.6 (0.5)	1.3 (0.5)	1.5 (0.7)	1.6 (0.9)	1.3 (0.4)	1.1 (0.4)
Zinc, mg	10.1 (3.1)	8.6 (3.2)	8.9 (3.1)	10.4 (5.6)	9.3 (2.9)	7.7 (2.9)

The values are represented as means (SD).

the trial failure is not credibly explained by wrong sample or wrong targets of treatment. However, in this regard we do need to note that the objective neuropsychological tests showed a more favorable pattern (Table 9), and if visual matching or seat movement had been designated the primary target (primary outcome measure) we would not be speaking of a failed trial.

A further possibility is incorrect administration of the treatment: Wrong dose, inadequate absorption, incomplete treatment, inadequate duration, or some manufacturing flaw. This line of examination does offer some plausible causes for the trial failure. There was enough question about capsule content and absorption based on lack of increase in blood levels that the capsules were quantitatively analyzed and a pharmacokinetic study was done. The quantitative analysis, on two different occasions, confirmed that the capsules (both placebo and active 15-mg capsules) contained the indicated amount of zinc. The pharmacokinetic study, however, showed only minimal increase of serum zinc and red cell 5′-nucleotidase at about an hour after ingestion. Two healthy control children showed a slightly greater rise in blood levels than the ADHD participants, but still not what was expected. Thus, although the capsules had the indicated amount of zinc, it does not seem to have been well absorbed even though the same preparation from the same manufacturer (different lots) had been shown in the past to be well absorbed and result in blood level rises. Doubling of the dose for the last dozen participants by giving an additional dose in the afternoon did result in improved outcome on some measures, notably neuropsychological outcomes and amphetamine dose. The duration of treatment (8 weeks monotherapy and 13 weeks adjunctive) does not seem inadequate in view of the fact that other studies have shown zinc repletion in <2 weeks. Also, Tables 6A and 6B do not reveal any difference between 8 versus 21 weeks of zinc with amphetamine.

Perhaps the most intriguing explanation is the possibility of deficient background nutrition, given that zinc works in the context

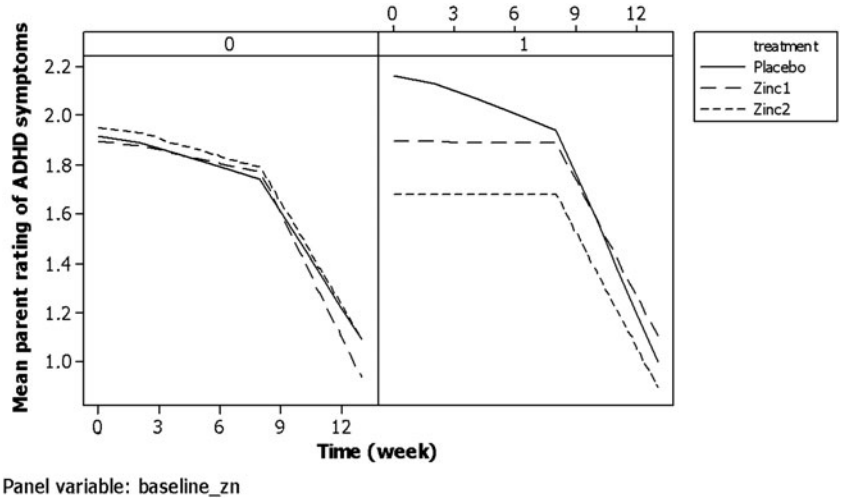

FIG. 2. Plot of parent rating of ADHD symptoms vs time (week). Moderator Graph. Time*group*baseline zinc interaction plot. Left panel is subgroup with low baseline zinc; right panel, those with baseline zinc (median split of sample). Dependent variable = parent rating of ADHD symptoms. Differences not statistically significant. ADHD = attention-deficit/hyperactivity disorder.

of multiple other nutrients. Most of the participants were not taking a daily multivitamin. However, the Food Frequency Questionnaire did not show alarming deficiencies of other nutrients. Nevertheless, increasing evidence is accumulating for the need to consider the total nutritional profile because of the interactions of various nutrients. For example, Bilici et al. (2004) noted that zinc was most effective in those with low levels of essential fatty acids, consistent with Arnold *et al*'s report (2000) that benefit of gamma-linolenic acid was restricted to those with borderline zinc status.

A related issue is that two of the positive Mideastern studies, one as monotherapy (Bilici et al. 2004) and one as adjunct to stimulant (Akhondzadeh et al. 2004), both used zinc sulfate rather than zinc glycinate. We chose glycinate to avoid gastrointestinal side effects and because it was well absorbed in previous work by one of us in different disorders. However, it is possible that the sulfate is required for the zinc to be effective in ADHD, or even that it may have been the sulfate rather than zinc that was the effective agent in the Mideastern trials.

Nevertheless, the most likely explanation for the success of the Mideast trials and failure of the one reported here is the difference in diets and soil. The Mideast is an area of endemic frank zinc deficiency, possibly related at least in part to high consumption of unleavened whole-grain bread and beans, in which phytates bind the zinc. The American diet is more variable. One of the purposes of this pilot study was to see if an American sample would show the same promising results found in the Mideast. It did not.

If this latter explanation is valid, it underscores the importance of context, location, and sample representativeness in a clinical trial. Site differences are not unusual in randomized clinical trials, but as long as there are not site-by-treatment interactions, that is not a concern. In this case, a multi-site trial might have shown a site-by-treatment interaction between an American site and a Mideastern site. The dietary differences also underscore the issue of interactions among nutrients.

Limitations of this study include all the possible sources of type II error described above. The sample was relatively small for a randomized clinical trial, as expected in an exploratory pilot study. Generalizability was limited by the sample representing only one locality and by the exclusion for disorders requiring pharmacotherapy other than stimulant. However, the latter exclusion is common in ADHD clinical trials, and only 2 of 59 screened potential participants were excluded on that basis. Another limitation common to randomized trials is self-selection of participants who are willing to take a chance on receiving placebo, and this may have been more important in a trial with eight possible weeks of placebo, a bit longer than most ADHD pharmacological trials. Conceivably the duration of placebo could have selected for milder severity; however, the baseline severity level was comparable to other randomized trials, such as the MTA (MTA Cooperative Group 1999). Only one zinc preparation was tried, also limiting generalizability. The failure to standardize other micronutrient intake to insure appropriate background nutrition, and the failure to consider interaction with essential fatty acids were further limitations.

Conclusion

Although this study does not support zinc supplementation, either alone or with stimulant, as treatment for ADHD in American children, the nagging questions about absorption and the importance of the anion (sulfate vs. glycinate), along with the paucity of zinc deficiency in the sample, the failure to insure other interacting micronutrients, the provocative neuropsychological test results, the upholding of the dosage hypothesis, and the suggestive moderator graph all leave considerable doubt about accepting the negative results. One has to wonder what would happen in an American sample selected for marginal zinc status treated with zinc sulfate in doses used by Bilici et al. (150 mg, equivalent to about 40 mg elemental zinc), or at least the 30 mg/day of zinc shown to be safe for 8 weeks in this study, and given a standard background mix of micronutrients, including essential fatty acids. The safety data do not indicate any great concern about larger doses up to 8 weeks, although the copper superoxide dismutase suggests a hint of interference with copper absorption (not upheld by ceruloplasmin assay). There was no apparent effect on ferritin levels from 30 mg/day of elemental zinc for 8 weeks. There was not an appreciable difference in clinical AE rates between placebo and zinc or between 30 mg/day and 15 mg/day of zinc. Therefore, it would be desirable to explore the situation further, with a different zinc preparation, higher doses, a sample selected for low zinc, insurance of background micronutrients via a standard daily multivitamin/mineral (without zinc), and perhaps with objective

neuropsychological tests as primary outcome, before discarding zinc as a potential treatment for ADHD in American patients.

Regardless, the possibility of zinc supplementation reducing the optimal dose of stimulant by over a third, shown in this pilot study despite the limitations described, merits further study. Given the concerns about long-term stimulant exposure, such a reduction could contribute immensely to public health. The principle has already been established for behavioral treatment, which can reduce the dose of stimulant required for a given effect, but is more expensive, difficult, and not readily available to all. If zinc could accomplish the reduction of stimulant dose, it would be cheaper, easier, and available to everyone.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent official views of the National Institutes of Health or the National Center For Research Resources.

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References

Agget PJ, Harries JT: Current status of zinc in health and disease states. Arch Dis Child 54:909–917, 1979.

Akhondzadeh S, Mohammadi MR, Khademi M: Zinc sulfate as an adjunct to methylphenidate for the treatment of attention deficit hyperactivity disorder in children: A double blind and randomized trial [ISRCTN64132371]. BMC Psychiatry 4:9, 2004.

Aman MG: Applications of computerized cognitive-motor measures to the assessment of psychoactive drugs. In The Assessment of Cognitive Function in Epilepsy, edited by WE. Dodson, M. Kinsbourne, B. Hiltbrunner. New York: Demos Publications, 1991, pp 69–96.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Washington, DC: American Psychiatric Association, 1994.

Arnold LE, Pinkham SM, Votolato N: Does zinc moderate essential fatty acid and amphetamine treatment of attention-deficit/hyperactivity disorder? J Child Adolesc Psychopharmacol 10:111–117, 2000.

Arnold LE, Votolato NA, Kleykamp D, Baker GB, Bornstein RA: Does hair zinc predict amphetamine improvement of ADHD/hyperactivity? Int J Neurosci 50:103–107, 1990.

Bales CW, DiSilvestro RA, Currie K, Plaisted CS, Joung H, Galanos AN, Lin P: Marginal zinc deficiency in older adults: Responsiveness of zinc status indicators. J Am Coll Nutr 13:455–462, 1994.

Bertrand A, Buret J: A one-step determination of serum 5'-nucleotidase using a centrifugal analyzer. Clin Chim Acta 119:275–284, 1982.

Bilici M, Yildirim F, Kandil S, Bekaroglu M, Yildirmis S, Deger O, Ulgen M, Yildiran A, Aksu H: Double-blind, placebo-controlled study of zinc sulfate in the treatment of attention-deficit/hyperactivity disorder. Prog Neuropsychopharmacol Biol Psychiatry 28:181–190, 2004.

Bekaroglu M, Yakup A, Yusof G, Orhan D, Hilal M, Erol E, Caner K: Relationships between serum free fatty acids and zinc and ADHD. J Child Psychol Psychiatry 37:225–227, 1996.

Best K, DiSilvestro RA: Signs of poor copper status in cystic fibrosis patients which are not reversible by six weeks of oral copper supplementation. FASEB J 15:A1097, 2001.

Black MM: Zinc deficiency and child development. Am J Clin Nutr 68(2 Suppl):464S–469S, 1998.

Blostein-Fujii A, DiSilvestro RA, Frid D, Katz C, Malarkey W: Short term zinc supplementation in type II diabetic women: Effects on plasma 5'-nucleotidase activities, insulin-like growth factor levels & lipoprotein oxidation rates in vitro. Am J Clin Nutr 66:639–642, 1997.

Conners CK: Conners Rating Scales—Revised. Technical Manual. North Tonawanda, NY: Multi-Health Systems, Inc., 2001.

DiGirolamo AM, Ramirez-Zea M, Wang M, Flores-Ayala R, Martorell R, Neufeld LM, Ramakrishnan U, Sellen D, Black MM, Stein AD: Randomized trial of the effect of zinc supplementation on the mental health of school-age children in Guatemala. Am J Clin Nutr 92:1241–50, Epub ahead of print Sept. 29, 2010.

Eck LH, Klesges RC, Hanson CL, White J: Reporting retrospective dietary intake by food frequency questionnaire in a pediatric population. J Am Diet Assoc 91:606–608, 1991.

Fristad MA, Emery B, Beck SJ: Use and abuse of the children's depression inventory. J Consult Clin Psychol 65:699–702, 1997.

Garvey JS, Vander Mallie RJ, Chang CC: Radioimmunoassay of metallothioneins. Meth Enzymol 84:121–138, 1982.

Golub MS, Takeuchi PT, Keen CL, Hendrickx AG, Gershwin ME: Activity and attention in zinc-deprived adolescent monkeys. Am J Clin Nutr 64:908–915, 1996.

Halas ES, Sandstead HH: Some effects of prenatal zinc deficiency on behavior of the adult rat. Pediatr Res 9:94–97, 1975.

Hunter DJ, Rimm EB, Sacks FM, et al.: Comparisons of measures of fatty acid intake by subcutaneous fat aspirate, food frequency questionnaire, and diet records in a free-living population of US men. Am J Epidemiol 135:418–427, 1992.

Joung H, DiSilvestro RA: Changes in zinc and copper metabolism after trauma. Nutr Res 18:693–701, 1997.

Kovacs M: The Children's Depression Inventory (CDI). North Tonawanda, NY: Multi-Health Systems, 1992.

Kozielec T, Starobrat-Hermelin B, Kotkowiak, L: Deficiency of certain trace elements in children with hyperactivity (Polish). Psychiatr Pol 28:345–353, 1994.

Lepping P, Huber M: Role of zinc in the pathogenesis of attention-deficit hyperactivity disorder: Implications for research and treatment. CNS Drugs 24:721–728, 2010.

Maes M, De Vos N, Demedts P, Wauters A, Neels H: Lower serum zinc in major depression in relation to changes in serum acute phase proteins. J Affect Disord 56:189–194, 1999.

MTA Cooperative Group: A 14-Month randomized clinical trial of treatment strategies for attention-deficit/hyperactivity disorder. Arch Gen Psychiatry 56:1073–1086, 1999.

Psychopharmacology Bulletin: Special feature: Rating scales and assessment instruments for use in pediatric psychopharmacology research. Psychopharmacol Bull 21(4), 1985.

Sandstead HH, Fosmire GJ, Halas ES, Jacob RA, Strobel DA, Marks EO: Zinc deficiency: Effects on brain and behavior of rats and Rhesus monkeys. Teratology 16:229–234, 1977.

Sprague RL, Sleator EK: Methylphenidate in hyperkinetic children: Differences in dose effects on learning and social behavior. Science 198:1274–1276, 1977.

Sprague RL, Toppe LK: Relationship between activity level and delay of reinforcement. J Exp Psychol 3:390–397, 1966.

Starobrat-Hermelin B: The effect of deficiency of selected bioelements on hyperactivity in children with certain specified mental disorders. Ann Acad Med Stetinensis 44:297–314, 1998.

Stein AD, Shea S, Basch CE, Contento IR, Zybert P: Consistency of the Willett semiquantitative food frequency questionnaire and 24-hour dietary recalls in estimating nutrient intakes of preschool children. Am J Epidemiol 135:667–677, 1992.

Swanson JM: School-based Assessments and Interventions for ADD Students. Irvine, CA: K.C. Publishing, 1992. Available at www .adhd..net

Swanson JM, Kraemer HC, Hinshaw SP, Arnold LE, Conners CK, Abikoff HB, Clevenger W, Davies M, Elliott, G, Greenhill, LL, Hechtman, L, Hoza, B, Jensen, PS, March, JS, Newcorn JH, Owens L, Pelham, WE, Schiller E, Severe, J, Simpson S, Vitiello, B, Wells, CK, Wigal, T, Wu, M: Clinical relevance of the primary findings of the MTA: Success rates based on severity of ADHD and ODD symptoms at the end of treatment. J Am Acad Child Adolesc Psychiatry 40:168–179, 2001.

Thomas EA, Bailey LB, Kauwell GB, Lee DY, Cousins RJ: Erythrocyte metallothionein response to dietary zinc in humans J Nutr 122:2408–2414, 1992.

Thompson RPH: Assessment of zinc status. Proc Nutr Soc 50:19–28, 1991.

Toren P, Sofia E, Sela BA, Wolmer L, Weitz R, Dov I, Koren S, Reiss A, Weizman R, Laor N: Zinc deficiency in ADHD. Biol Psychiatry 40:1308–1310, 1996.

Uckardes Y, Ozmert EN, et al.: Effects of zinc supplementation on parent and teacher behaviour rating scores in low socioeconomic level Turkish primary school children. Acta Paediatr 98:731–736, 2009.

Wakimoto P, Block G. Dietary intake, dietary patterns, and changes with age: An epidemiological perspective. J Gerontol 56 Spec No 2:65–80, 2001.

Ward NI: Assessment of chemical factors in relation to child hyperactivity. J Nutr Environ Med (Abingdon) 7:333–342, 1997.

Ward NI, Soulsbury KA, Zettel VH, Colquhoun ID, Bunday S, Barnes B: The influence of the chemical additive tartrazine on the zinc status of hyperactive children: A double-blind placebo-controlled study. J Nutr Med 1:51–58, 1990.

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