Oxidized LDL, diet, and natural antibodies

Dear Sir:

Zock and Katan (1) gave a good analysis in their recent editorial of the diet–LDL oxidation–coronary artery disease hypothesis. We would like to expand on the theoretical pathways involved in the oxidation hypothesis. Our theories are an offshoot of our recent finding of autoantibodies to cholesterol oxides in healthy individuals (2). In an enzyme-linked immunosorbent assay, these natural antibodies to cholesterol oxides bound to target antigens that included 7-ketocholesterol, cholesterol epoxide, 7-hydroxycholesterol, and 19-hydroxycholesterol. These cholesterol oxides represent major components of LDL that are modified by oxidation (2, 3). Because natural antibodies have been postulated to have an "immunohousekeeping" function (4), these antibodies to cholesterol oxides may be involved in the immunophysiologic clearance of oxidized LDL and aged cell membranes that contain a substantial amount of cholesterol.

Previously, we showed the common occurrence of natural antibodies to phospholipids including cardiolipin and phosphatidylserine (5). Antibodies to phospholipids were recently shown to cross-react with oxidized LDL (6). Although the immunologic origin of natural antibodies to phospholipids is still unknown, it was interesting to note that the amounts of these antibodies could be affected by diet in an experimental mouse model of autoimmune disease (7). Presumably, the same dietary factors influence the natural antibody populations.

We thus propose that in addition to the current scheme of atherogenetic events as summarized by Zock and Katan, natural antibodies to phospholipids, cholesterol oxides, or both—and by extension, oxidized LDL—may also serve to modulate any pathobiologic effects of oxidized LDL on the endothelium, platelets, and macrophages. The amount and activity of circulating oxidized LDL could therefore be controlled by regulatory mechanisms involving endogenous and exogenous antioxidants as well as natural antibody activity (**Figure 1**). The postulated protective role of natural antibodies also extends the spectrum of effectors that have been described in the immunologic control of atherogenesis (8).

Hwee-Ming Cheng

Department of Physiology
Faculty of Medicine
Universiti Malaya
Kuala Lumpur
Malaysia
E-mail: chenghm@medicine.med.um.edu.my

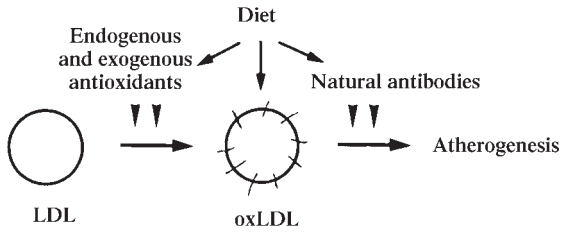

FIGURE 1. Potential influence of antioxidants and natural antibodies on the concentration and cellular effects of oxidized LDL (oxLDL). Besides containing antioxidants and other factors, the diet may also modulate the amount of natural antibodies to oxLDL.

Kalyana Sundram

Department of Nutrition
Palm Oil Research Institute
Selangor
Malaysia

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Reply to H-M Cheng and K Sundram

Dear Sir:

Chen and Sundram suggest that 7-ketocholesterol, cholesterol epoxide, and other cholesterol oxides represent major components of oxidized LDL, and that circulating autoantibodies to such cholesterol oxides may help to clear oxidized LDL in vivo. Treatment

Am J Clin Nutr 1999;70:104–13. Printed in USA.

of LDL with oxidizing agents in vitro indeed produces cholesterol oxides (oxysterols). However, the amounts of such cholesterol oxides that circulate in LDL in vivo may have been overestimated in early studies including our own (1) because significant amounts of oxysterols may be formed by cholesterol autoxidation during fractionation of plasma (2, 3). We ourselves attempted to discriminate oxysterols formed in vivo and in vitro by adding deuterated cholesterol ($m + 7$) after blood sampling. After subsequent plasma fractionation (4), the concentrations of deuterated cholesterol α-epoxide, deuterated 7-β-hydroxycholesterol, and deuterated 7-ketocholesterol were similar to those of the natural cholesterol oxidation products supposedly already present in vivo (47, 17, and 31 nmol deuterated products/L compared with 62, 23, and 31 nmol natural cholesterol oxides/L, respectively) (van de Bovenkamp and Hectors, unpublished observations, 1996).

This confirms that some of the oxysterols found in plasma may be artifacts produced by autooxidation in vitro. We therefore felt that the evidence for a role of circulating cholesterol oxides in human atherosclerosis was too weak to include in our editorial. The importance of the autoantibodies described by Chen and Sundram is even more speculative.

Martijn B Katan
Peter L Zock
Peter van de Bovenkamp

Wageningen Centre for Food Sciences and
Division of Human Nutrition and Epidemiology
Wageningen University
Bomenweg 2
6703 HD Wageningen
Netherlands

Magda Hectors

Division of General Internal Medicine
University Hospital Nijmegen
PO Box 9101
6500 HB Nijmegen
Netherlands

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Prebiotics or probiotics for lactose intolerance: a question of adaptation

Dear Sir:

Recent publications downplaying the clinical significance of lactose intolerance notwithstanding, the study by Saltzman et al (1), which failed to show clinical improvement in subjects with lactose intolerance after treatment with *Lactobacillus acidophilus* BG2FO4, raises some interesting questions regarding colonic bacterial adaptation. Lactose and lactulose have been reported to improve lactose intolerance in formal studies (2, 3). The areas under the curve (AUCs) for breath hydrogen and symptom scores diminished and fecal β-galactosidase concentrations increased after a period of exposure to either lactose or lactulose for 8 (1) to 16 (3) d. Furthermore, Hertzler et al (4) showed that the decrease in the AUC for breath hydrogen was due to decreased production and not to increased consumption of hydrogen. Because increased fecal β-galactosidase concentrations would theoretically suggest an increased metabolic capacity to digest lactose, an observation of decreased hydrogen production is an unexpected finding. Thus, in studies using prebiotics, fecal β-galactosidase may be more of a marker than a functional component of an expanded population of lactic acid bacteria.

Lactobacilli appear to behave differently depending on the species. Although changes in fecal bacterial enzymes are observed when lactobacilli are fed (5, 6), measured alterations in the AUCs for breath hydrogen vary with species (7). For example, Lin et al (7) found that *L. bulgaricus* improved the AUC for breath hydrogen and symptoms, whereas *L. acidophilus* did not. β-Galactosidase characteristics, however, appeared similar with both species. Patients with a short bowel, but an intact colon, represent a natural example of functional bacterial colonic adaptation to carbohydrates. Briet et al (8) showed that such patients had already adapted to a challenge dose of lactulose compared with naïve, normal subjects. The triple feature of colonic adaptation (reduced AUC for breath hydrogen, improved symptoms, and elevated fecal β-galactosidase concentrations) was easily discerned (8). In such patients the predominant fecal flora were lactobacilli of different species, including *L. acidophilus* (9). On the basis of these observations, one can question whether a different species of lactobacilli might not have given better results than *L. acidophilus* BG2FO4, whether a longer period than 7 d of exposure to *L. acidophilus* BG2FO4 might improve results (eg, in patients with short-bowel syndrome), or whether the clinical expression of colonic bacterial adaptation depends on interactions among several types of bacteria.

In any event, one conclusion to be drawn from the review of the literature is that prebiotics may be more efficient than probiotics in both achieving colonic bacterial adaptation and affecting lactose intolerance. However, both methods may ultimately have beneficial effects on colonic disease (reviewed in 10).

Andrew Szilagyi

Division of Gastroenterology
Department of Medicine
The Sir Mortimer B Davis Jewish General Hospital
Montreal H3T 1E2
Quebec
Canada

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Dietary supplement or drug? The case for Cholestin

Dear Sir:

In his editorial about our recent article, "Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement" (1), Havel (2) focuses on the issue of whether Cholestin (Pharmanex, Simi Valley, CA) is a drug or dietary supplement, citing the position of the Food and Drug Administration, rather than on the merits of our recent double-blind clinical trial of a standardized, commercial dietary supplement. In addition, he takes issue with 2 important facts. First, he states that the dietary supplement Cholestin actually differs from the traditional red yeast used as a dietary staple in Asia, which is prepared by fermenting yeast on rice. His sole argument for this position is that Cholestin "is manufactured by growing a single strain of *M. purpureus* on rice under carefully controlled conditions that increase the statin content, which is monitored during production." This is incorrect. The strain is selected as one that produces a family of monacolins, one of which is lovastatin (monacolin K). The actual composition of a Cholestin capsule is only yeast and the rice on which the yeast was fermented. Of course, when one produces a food, it is usual to monitor the product for the content of marker substances to ensure the constancy of production methods. However, there was no attempt to increase the production of the monacolins during fermentation. Selecting a yeast strain is no different from selecting a particular strain of tomato to grow for sale as a food on the basis of its red color (or perhaps someday its lycopene content). This is an essential point for dietary supplements at the growing edge of nutrition. Supplements are not unpurified drugs, but are natural substances. Drugs are produced by crystallization and purification from plant sources; a significant proportion of all drugs are derived from plants. The effect on public health of affordable and safe dietary supplementation cannot be underestimated.

Havel's second factual misinterpretation is that the statin content of the supplement is 10 mg. In fact, the appropriate comparison is between monacolin K and lovastatin, of which there is only 5 mg per tablet. Therefore, the comparison of the cholesterol-lowering effects of the dietary supplement with those of 10 mg lovastatin, which was tested in a multicenter trial by Havel et al (3), is inappropriate. As a dietary supplement, this yeast contains ten monacolins (1), which may have significant cholesterol-lowering activity, and differs from lovastatin. The activity of these other substances, and this needs to be tested. Because this dietary supplement is based on a traditional Asian food, it is reasonable to assume that it is safe; and the Dietary Supplement Health and Education Act was specifically written to protect dietary supplement manufacturers from being required to conduct the expensive trials required of manufacturers of purified drugs (4). One reason costs are high in the US health care system is because drug testing is expensive. In fact, only small numbers of individuals in this country are currently taking cholesterol-lowering drugs—even individuals with cholesterol concentrations >6.2 mmol/L (240 mg/dL).

The need for an alternative to prescription drugs for the tens of millions of Americans with cholesterol concentrations between 5.2 and 6.2 mmol/L (200 and 240 mg/dL) is clear to me. As a physician, I am frequently faced with the dilemma of which treatment option is best for patients who have changed their diets and lifestyles optimally, but who still have undesirably high cholesterol concentrations. My only choice, other than natural remedies (5), is to prescribe drugs for my patients who have cholesterol concentrations below values for which these drugs are approved. Since the publication of Havel's editorial, the Federal District Court has ruled against the Food and Drug Administration, finding that Cholestin is a dietary supplement. I urge all nutritionists to become informed about the entwined scientific, public health, and legal issues concerned with dietary supplements so we can fulfill our important mission.

David Heber

UCLA Center for Human Nutrition
900 Veteran Avenue
Room 13-146 Warren Hall
Los Angeles, CA 90095-1742
E-mail: dheber@med1.medsch.ucla.edu

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Reply to D Heber

Dear Sir:

In his response to my editorial (1) concerning his recent article (2), Heber complains that I failed to deal with the merits of his

paper. The purpose of my editorial, however, was to call attention to the litigation between Pharmanex, Inc, Simi Valley, CA (the manufacturer of Cholestin, the supplement studied by Heber et al), and the US Food and Drug Administration (FDA). The litigation concerns the administrative FDA ruling that Cholestin is a drug and not a food supplement under the Dietary Supplement Health and Education Act of 1994 (3). Cholestin contains sufficient statin compounds, principally monacolin K (also known as lovastatin) to appreciably alter cholesterol metabolism and lower plasma cholesterol concentrations, as documented by Heber et al (2).

Heber takes issue with 2 "facts" that are major points in my editorial. The first concerns the issue of litigation. Heber believes that my statement that "Cholestin is manufactured…under carefully controlled conditions that increase the statin content, which is monitored during production," is "incorrect." Rather, he maintains that "the strain [of *Monascus purpureus*] is selected as one that produces a family of monacolins, one of which is related to lovastatin." My editorial referenced Public Docket no. 97P-0441, which is accessible on the World Wide Web. The decision of the FDA contained therein is in a lengthy letter from William B Schultz, Deputy Commissioner for Policy of the FDA, to Stuart M Pape, an attorney for Pharmanex, Inc. The letter cites a company promotional document indicating that Pharmanex developed its own "proprietary process" in 1993 to make a red-yeast-rice product containing amounts of lovastatin that could "maximize red yeast's health-enhancing properties." The letter goes on to cite 3 ways in which this has been done.

"First, Pharmanex is deliberately controlling temperature conditions during the manufacturing process to promote consistently high levels of lovastatin in Cholestin… Key factors for production [are] both temperature and oxygen tension." (The letter cites data indicating that little or no lovastatin is produced by *M. purpureus* at temperatures ≥30°C and that the optimum temperature for statin production is ≈25°C.) "Second, Pharmanex tracks the level of HMG-CoA [β-hydroxy-β-methylglutaryl-CoA] reductase inhibitors in Cholestin, of which lovastatin is the most abundant, during the production process. This tracking ensures significant levels of the drug in the final Cholestin product." (The letter points out that the HMG-CoA inhibitors in Cholestin are secondary metabolites, the concentrations of which "do not follow fungal growth," contrary to the company's statement that it does the monitoring as "a biochemical marker with which to monitor the level of yeast.") "Third, Pharmanex's careful selection of a particular fungal strain to manufacture Cholestin indicates that the company seeks to manufacture lovastatin. Only select strains of Monascus fungus are capable of producing lovastatin." The letter goes on to state that "Pharmanex itself has admitted that Cholestin is not traditional red yeast rice." It points out that traditional red yeast rice is made from a mixture of strains, and, notably, that traditional red yeast rice contains little or no material of the statin class. Data contained in the letter indicate that the content of statins produced by *M. purpureus* is inversely related to the content of red pigment. Thus, Cholestin would not be suitable for the traditional purposes of red yeast rice.

My own calculations show that the average content of lovastatin in 33 samples of traditional red yeast rice tested by the FDA was 3% of the amount contained in Cholestin (30 samples contained no detectable amount). Finally, the letter includes the following summary statement: "FDA does not believe that…Cholestin is traditional red yeast rice. This conclusion is supported by evidence in the record indicating that: (1) Cholestin was developed in l993 pursuant to a proprietary process, while traditional red yeast rice has existed for centuries; (2) traditional red yeast rice comes from a mixture of fungal strains while Cholestin is manufactured from only one fungal strain; (3) traditional red yeast rice contains pigments, which indicates that the traditional product does not contain significant levels of lovastatin, as does Cholestin; (4) traditional red yeast rice is fermented at temperatures that preclude the production of significant levels of lovastatin, such as those found in Cholestin; and (5) test results indicate that traditional red yeast rice on the market today does not contain lovastatin at the levels found in Cholestin, if at all." This evidence fully supports the statement in my editorial that is now challenged by Heber.

The decision of the FDA contained in Public Docket no. 97P-0441 was recently overturned by the US District Court for the District of Utah (4). The court based its decision on a particular interpretation of the meaning of terms in the Dietary Supplement Health and Education Act of 1994 and not on any distinction between Cholestin and traditional red yeast rice. I believe that the court's decision, if sustained, will materially affect the production and marketing of food supplements in this country.

Concerning the second "fact" that Heber takes issue with, he states that I misinterpreted the statin content of Cholestin and that my "comparison of the cholesterol-lowering effects of the dietary supplement with those of 10 mg lovastatin…is inappropriate." Heber erroneously states that a more appropriate comparison would be with "monacolin K, of which there is only 5 mg per tablet." Actually, there is 5 mg monacolin K in 4 tablets of Cholestin, which comprises about one-half of the total amount of statin compounds. However, my statement, "The amount of statins in 2.4 g Cholestin is 10 mg." is correct. Furthermore, I indicated that "If the other statin compounds in Cholestin are equal in activity to lovastatin, the total complement of reductase inhibitors evidently accounts for most of the product's cholesterol-lowering action." I stand by this statement.

Heber states, "Because this dietary supplement is based on a traditional Asian food, it is reasonable to assume that it is safe…" However, the safety of the several monacolins in Cholestin other than lovastatin is unknown. Furthermore, lovastatin has low bioavailability, related to intestinal CYP3A enzymes, which are subject to inhibition not only by several other drugs, but also by grapefruit juice. In a recent study, prior ingestion of large amounts of grapefruit juice increased serum concentrations of lovastatin 5–20-fold (5). Interactions between monacolins and grapefruit juice and several drugs have the potential for serious adverse consequences, such as rhabdomyolysis, for persons taking Cholestin (but not traditional red yeast rice). I sympathize with Heber's concern about his patients, but I do not believe that a preparation that contains significant amounts of HMG-CoA reductase inhibitors should be available to people who may not be under medical supervision.

Richard Havel

University of California, San Francisco
Cardiovascular Research Institute
505 Parnassus Avenue, L1337
San Francisco, CA 94143-0130
E-mail: havelr@itsa.ucsf.edu

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Dietary fat and obesity

Dear Sir:

Willett's (1) recent editorial points out the need for long-term studies (≥1 y) to determine whether diets low in fat promote weight loss. As an example of a low-fat dietary intervention trial that does not support the hypothesis that graded weight loss results from a reduction in dietary fat as proposed by Bray and Popkin (3), he also cites the study by Knopp et al (2). This study failed to show an association between increasing weight loss and a progressively lower percentage fat intake (range: 22–28%). However, we emphasize that all groups undergoing dietary fat restriction in this study lost significant amounts of weight compared with baseline (range: 2–3 kg). This observation supports the conclusion that dietary fat restriction promotes sustained weight loss.

Several other studies lasting ≥1 y showed modest, but significant, sustained weight loss. Bray and Popkin included data from studies by Sheppard et al (4) and Siggaard et al (5) in their analysis. In another study, 14 subjects who lowered their fat intake to 21% of total energy intake for 1 y decreased their body weight by an average of 6.9 kg (6). More recently, in a 1-y follow-up study that included a control cohort, dietary fat restriction resulted in a significant decrease (from baseline) in fat intake from 28.4% to 22.7% of energy and in body weight from 69.6 to 67.2 kg (7). In the control group, no change in either of these variables was found at follow-up.

Those studies that measured energy intake showed that weight loss was associated with a spontaneous sustained reduction in daily energy intake (2, 4, 7). The fact that changes in the macronutrient content of the diet (lowering fat and increasing carbohydrate intakes) led to reductions in energy intake and long-term weight loss offers potentially important insight into the physiology of weight regulation (8, 9).

Dietary fat intake is only one environmental factor that affects the genetic expression of obesity. Therefore, it is not unexpected that dietary fat restriction results in a variable amount of weight loss, depending on one's genetic background. Schaefer et al (10) showed that when the subjects in their study switched from a high-fat to an ad libitum low-fat diet, they experienced a wide range of weight changes, from a gain of 1.5 kg to a loss of 13 kg, with an average loss of 3.3 kg. The notion of a low dietary fat "responder" being someone who loses >5% of their initial body weight and "nonresponders" being those who lose no weight is similar to other diseases whose expression is the result of gene-environment interactions. For instance, salt restriction may reduce blood pressure in up to 30–50% of subjects with "salt-sensitive" hypertension. Another example is subjects with hypercholesterolemia who are typically described as "dietary responders" if cholesterol concentrations decrease with restriction of dietary cholesterol and fat and as "nonresponders" if concentrations do not change. The above-mentioned studies support the view that dietary fat restriction in combination with regular aerobic exercise is sound advice that caregivers can give to their obese patients for the attainment of modest weight loss.

Jonathan Q Purnell
Robert H Knopp
John D Brunzell

University of Washington School of Medicine
Division of Metabolism, Endocrinology, and Nutrition
Box 356426
Seattle, WA 98195
E-mail: purnell@u.washington.edu

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Reply to JQ Purnell, RH Knopp, and JD Brunzell

Dear Sir:

Purnell et al have interpreted the fine study by Knopp et al (1) strangely and have ignored the very reason for conducting randomized trials—to account for factors other than the specific intervention that might change over time and thus influence the outcome being evaluated. The reason for conducting such a ran-

domized trial is to compare the change in the treatment group with the change in the parallel, control group and not to evaluate the significance of changes within groups. Important strengths of their study were that a large number of subjects were randomly assigned to receive 1 of 4 fat intakes and that each group received dietary counseling and monitoring. This allowed valid comparisons of the effects of different fat intakes on changes in weight. The fact that all groups lost ≈3 kg indicates strongly that the percentage of energy from fat, over the range studied, did not influence body weight and that some other factor common to all 4 groups did. Purnell et al stated that this common factor was the reduction in fat intake in all groups compared with baseline, even though weight reduction was not related to the decrease in fat intake. Although the common factor cannot be proven directly from this study, the Hawthorne effect is a more likely explanation. Specifically, that raising ones consciousness about food intake combined with intensive support, counseling, and feedback will result in a modest weight loss regardless of the percentage of energy from fat in the diet. Indeed, dietitians have long known that careful recording and monitoring of dietary intakes is an important component of weight control.

The finding of an effect of intervention unrelated to fat intake by Knopp et al highlights the methodologic shortcomings of most randomized trials of fat reduction and emphasizes the desirability of including a control group with a similar intensity of intervention in such studies. For example, interventions aimed at reducing total energy or carbohydrate intakes would be appropriate comparison strategies. The other studies cited by Purnell et al lacked such a control group. For example, in the trial by Sheppard et al (2), one group was given intensive instruction in fat reduction followed by careful monitoring via weighed food records, but the control group received no such intervention; the differences in the changes in weight between the 2 groups were −2.6 kg at 1 y and −1.8 kg at 2 y. The small number of studies on the effects of fat reduction on body weight in which the control group did receive intervention comparable with that of the treatment group showed minimal or no effects on body weight: none in Knopp et al's study (1) when properly analyzed, a loss of 1.4 kg in the study by Jeffery et al (3), and a loss of 0.8 kg in the National Diet Heart Study (4). Thus, the best evidence from long-term studies involving control groups with a similar level of intervention intensity show extremely little if any effect of the percentage of energy from fat on body weight. However, even if the more optimistic but biased assumption of a 2–3-kg effect were accurate, this is still an imperceptible and clinically unimportant change for an overweight or obese person. If the proponents of low-fat diets were more candid about the weight loss expected from a major change in diet, there would likely be few "takers," but less disillusionment and loss of credibility for the nutrition community. Patients prescribed a low-fat diet should be informed that such a diet is likely to increase serum triacylglycerol and reduce HDL-cholesterol concentrations (5), which are associated with a higher risk of coronary artery disease.

Purnell et al cite the findings of Schaefer et al's study (6) to suggest that changes in weight may vary in persons consuming low-fat diets, possibly because of genetic differences. However, this study lasted only 12 wk and longer-term evidence suggests that these weight changes would not be maintained. Variations in weight changes in response to changes in the mix of macronutrients are indeed possible, but genetic modifiers are yet to be identified. Because the average effect on body weight of a reduction in fat intake is so small in the longer-term studies conducted to date, if there is a common subgroup that responds with a major weight reduction, we would also need to hypothesize another subgroup that responds with weight gain.

Anyone would agree with Purnell et al that regular exercise will benefit most patients, but available evidence strongly suggests that a focus on energy intakes from fat with no regard to energy intake from carbohydrate will have little effect on body weight. Moreover, there is overwhelming evidence from prospective studies (7) and randomized trials (8) that replacement of saturated and *trans* fats with unsaturated fat in the diet will substantially reduce the risk of coronary artery disease, but that replacement of fat with carbohydrate in the diet will have little if any effect (9). The misguided focus on a reduction of dietary fat per se to reduce body weight has resulted in a lost opportunity to have a major effect on the most important cause of death in Western countries.

Walter C Willett

Department of Nutrition
Harvard School of Public Health
665 Huntington Avenue
Boston, MA 02115

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Reply to JQ Purnell, RH Knopp, and JD Brunzell

Dear Sir:

We concur with the comments of Purnell et al regarding our recent study (1). We are glad that other responsible scientists are also concerned about the problem of dietary fat and obesity, which

is a complex and important issue that deserves serious review.

George A Bray

Pennington Biomedical Research Center
Lousiana State University
6400 Perkins Road
Baton Rouge, LA 70808-4124
E-mail: brayga@mhs.pbrc.edu

Barry M Popkin

University of North Carolina
University Square
123 West Franklin Street
Chapel Hill, NC 27516

REFERENCE

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Improving study design

Dear Sir:

The recent article by Kikafunda et al (1) prompts me to relate an event that occurred ≈30 y ago when Mattie Ray Spivey-Fox, a highly respected basic scientist in nutrition, gently informed a group of clinical investigators that their research design was faulty. Primary human zinc deficiency had been characterized under metabolic ward conditions (2–4). When investigators took the new knowledge to the field, however, they were unable to replicate the observations (5–7). James Halsted organized a meeting to review the problem and Spivey-Fox was one of the attendees. She listened to the presentations and interpretations politely and then proposed a change in research design that provided the subjects with other potentially limiting nutrients. Her proposal was based on the fact that natural conditions seldom result in one deficiency at a time. Implementation of her suggestion resulted in the successful demonstration of improved growth of Iranian schoolboys with zinc repletion (8). The study by Kikafunda et al (1) might have been improved by design changes similar to those suggested by Spivey-Fox.

Harold H Sandstead

University of Texas Medical Branch at Galveston
Department of Preventive Medicine and Community Health
Ewing Building
700 Harborside Drive
Galveston, TX 77555-1109
E-mail: hsandste@utmb.edu

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Reply to HH Sandstead

Dear Sir:

Our study (1) was concerned with the effects of zinc supplementation on the growth and infection rate of 153 Ugandan children in 3 nursery schools. The children, in whom the prevalence of stunting was high, were randomly assigned to receive either fruit juice with 10 mg supplemental Zn/d or fruit juice with placebo on each school day for 8 mo.

A positive growth response was shown for children in only 1 of the 3 schools. This school catered to children of families with moderate incomes. In the 2 schools affordable to families of lower socioeconomic status, the children showed no growth response to zinc supplementation. We suggested that a deficiency of nutrients other than zinc may have accounted for the lack of response in these 2 schools and in similar studies that reported a lack of growth response to zinc supplementation.

The study by Ronaghy et al (2) mentioned by Sandstead involved 49 Iranian boys aged 13 y and bears some similarities to ours, but also some important differences. We were interested at the outset in determining whether, within the context of the diet consumed by young children in Uganda, a modest daily zinc supplement would be effective in combating the exceptionally high prevalence of stunting in that country. Hence, unlike Ronaghy et al, we did not consider adding supplements of energy and protein in an attempt to ensure dietary adequacy of macronutrients. Even if we had done so, as these authors pointed out for the children in their own study, there would have been no guarantee that the modest supplement of 127 kcal energy/d (531 kJ/d) used by Ronaghy et al would have resulted in an adequate energy intake for all the malnourished children in our study.

In hindsight, a design feature of the Iranian study that would have improved our Ugandan study was that all children were given a supplement of nonzinc vitamins and minerals to ensure that dietary targets for these nutrients were met during zinc supplementation. However, our findings were unexpected. We had not been alerted to the importance of nonzinc nutrient status in the growth response of children to zinc supplementation from previous reports.

In the Iranian study, all children were provided with a full spectrum of nonzinc vitamins and minerals, except magnesium. If nutrients are administered in future studies, it would be prudent to include magnesium. This is because magnesium and zinc deficiencies are type 2 deficiencies (3), sharing the following common features: *1*) there are no body stores of the nutrients, *2*) the body avoids tissue desaturation and uses conservation mechanisms early in the deficiency, and *3*) in children, reduction of growth rate is an adaptive mechanism to reduce demands for the nutrients. Magnesium intakes fail to reach nutritional targets in many children (4) and adults (5) in the United Kingdom and other Western countries and a similar situation is likely in the diet of young children fed refined cereals such as maize, which is eaten widely in Uganda. Unfortunately, few studies of malnourished populations have focused on this nutrient.

I am grateful to Sandstead for highlighting this issue. Clearly, more research is warranted to determine the value of zinc supplementation in the growth of children in studies designed to ensure the absence of deficiencies of other vitamins and minerals, including magnesium.

Ann F Walker

Hugh Sinclair Unit of Human Nutrition
Department of Food Science and Technology
The University of Reading
Whiteknights, Reading RG6 6AP
United Kingdom
E-mail: a.f.walker@afnovell.reading.ac.uk

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Single-nutrient interventions with zinc

Dear Sir:

The recent article by Kikafunda et al (1) raises several issues about oral zinc supplementation in free-living populations. In general, such an intervention might *1*) determine whether zinc deficiency is extant in a population, *2*) determine whether zinc is a limiting factor in growth, *3*) determine to what extent zinc nutriture is related to neurophysiologic performance, and *4*) investigate the antiinfective properties of zinc supplementation, or fulfill any combination of these motives. It appears that the study by Kikafunda et al embodied at least 3 of these.

Sandstead (2), in his landmark paper published in 1973, stated that whether a condition or abnormality was related to zinc deficits was best gauged by how the condition or abnormality responded (improved) to the administration of oral zinc. Several investigators, including ourselves, have followed this suggestion with oral zinc supplementation of free-living populations. Indeed, the Uganda-based researchers commented on our previous study in Guatemalan schoolchildren (3) and the study by Bates et al (4) in Gambian infants as examples of previous studies in which protracted administration of oral zinc in a randomized, placebo-controlled design led to changes in body composition but not to any increase in linear growth. That more data on zinc supplementation and body composition are not available is not because of a proven lack of effect, but rather because researchers have not looked at this association.

The critical issue, recognized by Kikafunda et al (1), is whether zinc, in the single-nutrient experimentation used, is or is not the first-limiting nutrient. Kikafunda et al found a weight-gain response in the school with the better-off student body, commenting: "The children from the school with the highest socioeconomic status, and therefore a better nutritional background, responded significantly in weight gain to zinc supplementation whereas the children from the poorer schools did not…. This indicated that zinc was the limiting nutrient in the nutrition of the children with relatively better nutritional status, whereas those with poorer nutritional status were deficient in other nutrients that limited the response in zinc supplementation." They note a feature of our study in Guatemalan schoolchildren (3), namely that it began with a pretreatment phase with supplementation of essential micronutrients (excluding zinc) so as to better expose zinc as the only remaining deficiency. In none of the other community-based zinc supplementation studies reviewed by Brown et al (5) was the need for the remaining micronutrients covered simultaneously. Perhaps even greater growth would have been seen in some of these studies if concurrent micronutrient deficiencies had not limited the responses. Recently, Sandstead et al (6) conducted a zinc supplementation trial using this approach (ie, zinc, zinc plus micronutrients, and micronutrients alone) in Chinese children. These investigators found that the knee-height increase was significantly greater in the zinc-plus-micronutrients group than in the zinc-alone group.

The comments by Kikafunda et al (1) and the aforementioned considerations suggest that it is likely that different effects will be seen when zinc is given in supplemental doses if an individual *1*) is without any micronutrient deficits, *2*) is uniquely zinc deficient, or *3*) has multiple micronutrient deficiencies. This will lead to heterogeneous responses to zinc in free-living populations because all 3 conditions will likely coexist to different degrees.

So what do we accomplish and what might we provoke in free-living populations when we apply oral zinc as a single nutrient in a prolonged intervention? This becomes even more imposing a question with emerging evidence of the antiinfective potential of zinc in preventing and shortening diarrhea, in combating respiratory infections, and even in reducing mortality in malaria (7). We can reverse zinc deficiency if zinc is the limiting nutrient; if the situation is one of multiple micronutrient deficiencies, however, we may fail to achieve the desired effect and instead create a nutrient imbalance.

There are clearly serious programmatic and policy implications: single-nutrient interventions might be avoided in favor of balanced, multinutrient programs, even when the primary objec-

tive is to provide zinc. It is heartening that some of the agencies working to combat zinc deficiency are moving toward a similar conclusion. In this Journal, Alnwick (8) commented on the perspective of the United Nations Children's Fund (UNICEF) that intermittent combined dosing of iron, iodine, vitamin A, vitamin D, riboflavin, folic acid, and zinc is being considered for its feasibility. As more and more single-nutrient intervention research is conducted, it becomes clear that zinc-responsive deficits in body composition are widespread in developing countries. However, how clearly they are revealed in experimental trials, how efficiently they are addressed in public health nutrition, or how well-tolerated will be the chronic administration of oral zinc to prevent childhood infections may depend on the simultaneous redress of coexisting micronutrient deficiencies.

Noel W Solomons

Center for Studies of Sensory Impairment, Aging and Metabolism (CESSIAM)
Guatemala City 01011
Guatemala
E-mail: cessiam@tikal.net.gt

Manuel Ruz

Center for Human Nutrition
Faculty of Medicine
University of Chile
Santiago
Chile

Rosalind S Gibson

Department of Human Nutrition
University of Otago
Dunedin
New Zealand

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Reply to NW Solomons et al

Dear Sir:

I am pleased that our paper (1) highlighted the need to address all potential micronutrient deficiencies in future studies of zinc intervention. Because zinc is an integral part of so many enzymes, the activity of which is dependent on the presence of a range of micronutrients, it would not be surprising to find that the full nutrient potential of zinc is realized only when these micronutrients are adequately supplied in the diet.

With regard to multinutrient interventions, one nutrient that has received little attention is magnesium, which is likely to be low in the refined diets of many children in developing countries. I emphasize magnesium because its nutrition has many characteristics in common with that of zinc (eg, lack of body stores, multiplicity of roles, and growth cessation as an adaptive response to deficiency). Unfortunately, the relevancy of magnesium deficiency to human health is often overlooked. This is despite the fact that in most dietary surveys of those eating refined diets, as exemplified by the UK National Diet and Nutrition Survey (2, 3), magnesium emerges at the top of the list of nutrients for which persons (often the majority) in all age groups fail to reach dietary targets. It was reassuring that 100 mg Mg was included in the micronutrient supplement administered daily to both the zinc-supplemented and placebo groups of children in the Guatemalan study (4). This inclusion was unusual because magnesium is usually excluded from multinutrient supplements on the grounds that a meaningful daily supplement of the mineral would make the formulation too large to swallow in a once-daily tablet.

As Solomons et al indicate in their letter, the results of our study provide a possible explanation for the variable growth responses to zinc supplementation seen in previous studies of children. This now needs to be followed up with studies designed specifically to test the hypothesis that a full growth response to zinc occurs only in a state of repletion of other micronutrients. Dissecting the role of magnesium in the zinc growth response would be particularly interesting. Once a clear picture emerges for growth, other responses of zinc repletion could be examined similarly, including the immune response. I welcome the letter from Solomons et al and fully agree that our study further emphasizes the notion that a cautious approach should be taken to the use of single-mineral supplements in public health programs.

Ann F Walker

Hugh Sinclair Unit of Human Nutrition
Department of Food Science and Technology
The University of Reading
Reading RG6 6AP
United Kingdom
E-mail: a.f.walker@afnovell.reading.ac.uk

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