

The Mediterranean diet as prevention strategy for dementia as a multicausal geriatric syndrome

Dear Sir:

With great interest we read the article by Kesse-Guyot et al (1), which is presented as evidence against the beneficial long-term effects of the Mediterranean diet on cognitive aging. Our own research focuses on an Internet-mediated intervention on lifestyle changes, including improving dietary habits (2). We acknowledge the great value of a long-term follow-up study on the Mediterranean diet. This long-term follow-up on cognition, linking it to the enormous global threat of dementia in our aging societies, is the strongest point of the study. However, the long-term gap in data collection is by far the weakest point of the study, which makes the data very hard to interpret and severely jeopardizes external validity for primary prevention in daily practice.

As previously indicated, the 13-y follow-up period of the large trial is impressive. However, we argue that the use of Mediterranean diet adherence measures from the initial 1994–1996 period combined with cognitive results from the 2007–2009 period is unreliable. At best, there is an 11-y gap between both measurements. Within these 11 y, dietary habits of the entire population could have undergone drastic changes. Given the fact that the unknown period is, at a minimum, 5 times longer than the period of measurement, the effects this probably has on cognitive aging patterns far outweigh the effects of the 2-y intervention period described in the article.

A very recent randomized trial on the Mediterranean diet in middle-aged persons with high cardiovascular risk in Spain showed a 0.70 (unadjusted hazard ratio with 95% CI: 0.53, 0.94) improvement in the incidence of cardiovascular endpoints over 5 y of follow-up with a Mediterranean diet enriched with olive oil or nuts (3). This is in line with several positive health outcomes that previously have been related to adhering to the Mediterranean diet. Kesse-Guyot et al (1) do not specifically take these cardio- and cerebrovascular endpoints into account in their study. We are curious about the positive effects the study's Mediterranean diet may have had on the onset of type 2 diabetes, high blood pressure, and cardiovascular diseases (4, 5). All of these factors have been firmly associated with the way our brain ages (6–8). Therefore, adherence to the Mediterranean diet mechanically is expected to have at least an indirect positive effect on cognitive aging patterns. This is in contrast to this article's conclusion that the "study does not support the hypothesis of a significant neuroprotective effect of a MedDiet on cognitive function." By failing to discuss this indirect effect of healthy nutrition on cognitive aging, Kesse-Guyot et al (1) oversimplify the complex problem at hand.

In conclusion, we find the long-term effects of the Mediterranean diet raised by Kesse-Guyot et al (1) very relevant but cannot share their opinion that their results provide compelling evidence that the Mediterranean diet does not provide a neuroprotective effect on cognitive function. Methodologic flaws in monitoring the intervention and the outcomes seriously weaken their lack of evidence for

effect, which should not be confused with evidence for the absence of effect. On the contrary, there are still strong arguments for long-lasting beneficial effects of the Mediterranean diet. First, there is increasing evidence that dementia among the elderly is not a monocausal neurological disease but a geriatric syndrome based on several component causes, including neurodegenerative cortical loss, disorders of cerebral circulation, nutritional deficiencies, and potentially many others (9). This, together with the strong recent findings on the beneficial effects of the Mediterranean diet on cardio- and cerebrovascular endpoints, strongly supports the hypothesis that great beneficial effects may be expected in populations that change their current Western diet to a nutritional lifestyle that seriously adopts the Mediterranean diet.

The authors declared no conflicts of interest.

*Teun Aalbers
Lia Baars
Marcel Olde Rikkert*

Department of Geriatric Medicine
Radboud University Nijmegen Medical Centre
PO Box 9101 6500 HB
Nijmegen Netherlands
E-mail: t.aalbers@ger.umcn.nl

REFERENCES

1. Kesse-Guyot E, Andreeva VA, Lassale C, Ferry M, Jeandel C, Hercberg S, Galan P; SU.VI.MAX 2 Research Group. Mediterranean diet and cognitive function: a French study. *Am J Clin Nutr* 2013;97:369–76.
2. Aalbers T, Baars MAE, Olde Rikkert MGM. Characteristics of effective Internet-mediated interventions to change lifestyle in people aged 50 and older: a systematic review. *Ageing Res Rev* 2011;10:487–97.
3. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Laperta J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013; 368:1279–90.
4. Domínguez LJ, Bes-Rastrollo M, de la Fuente-Arrillaga C, Toledo E, Beunza JJ, Barbagallo M, Martínez-González MA. Similar prediction of decreased total mortality, diabetes incidence or cardiovascular events using relative- and absolute-component Mediterranean diet score: the SUN cohort. *Nutr Metab Cardiovasc* 2012; March 6 (Epub ahead of print; DOI:10.1016/j.numecd.2011.10.009).
5. Esposito K, Maiorino MI, Ceriello A, Giugliano D. Prevention and control of type 2 diabetes by Mediterranean diet: a systematic review. *Diabetes Res Clin Pract* 2010;89:97–102.
6. Exalto LG, Whitmer RA, Kappelle LJ, Biessels GJ. An update on type 2 diabetes, vascular dementia and Alzheimer's disease. *Exp Gerontol* 2012; 47:858–64.
7. Sörös P, Whitehead S, Spence JD, Hackinski V. Antihypertensive treatment can prevent stroke and cognitive decline. *Nat Rev Neurol* 2013;9:174–8.
8. de la Torre JC. Cardiovascular risk factors promote brain hypoperfusion leading to cognitive decline and dementia. *Cardiovasc Psychiatry Neurol* 2012;2012:367516.
9. Strandberg TE, O'Neill D. Dementia—a geriatric syndrome. *Lancet* 2013; 381:533–4.

doi: 10.3945/ajcn.113.062232.

Reply to T Aalbers et al

Dear Sir:

We thank Aalbers et al for their letter with regard to our recent publication on midlife Mediterranean diet adherence and subsequent cognitive function (1).

In fact, as stated in the article, our aim was to explore the cross-time association between midlife dietary exposure (adherence to a Mediterranean diet) and subsequent cognitive function. Adherence to a Mediterranean diet was assessed by using repeated 24-h dietary records collected over a 2-y period. That time frame was chosen to assess “usual, typical diet,” as is often done in longitudinal epidemiologic studies. We would like to clarify that our study did not involve any intervention. In other words, we did not promote a Mediterranean diet (or any aspect thereof) in any way. Perhaps, a possible source of confusion was the fact that the SU.VI.MAX participants were recruited for an antioxidant intervention trial that was entirely unrelated to the study of Mediterranean diet adherence and cognition. The SU.VI.MAX protocol included the collection of self-reported data on dietary intake (via repeated 24-h dietary records), and those are the data used in the present study.

We agree that some major changes in dietary patterns might have occurred for some individuals (but not for the entire cohort) during the 13-y interval between the assessment of dietary exposure and the cognitive evaluation. However, we believe that such changes are less likely to occur in an observational context than in an intervention context.

We feel that another point that merits clarification is the fact that we did not have measures for, and thus did not assess, “cognitive aging” or “cognitive aging patterns.” As noted in the article, we assessed cognitive performance only at follow-up via a battery of 6 neuropsychological tests. As acknowledged in the Limitations section, the lack of baseline or cross-time cognitive performance assessment prevented the examination of cognitive decline. However, concerning the possibility of reverse causality, the inclusion of healthy volunteers in a long-term cohort focused on nutrition ensures a low probability of dietary habits modification due to cognitive impairment. Even though residual confounding is always possible, the extensive statistical adjustment also helped to limit the effect of preexisting differences in cognitive performance.

As discussed in our article, our prior work bears on cognitive performance studies with a similar design but with different dietary exposures of interest (2, 3). The presence of significant associations in those studies argues for explanations of the findings that go beyond the methodology. In turn, a feasible explanation for the lack of support for a beneficial role of adherence to a Mediterranean diet in cognitive performance is the fact that the level of adherence to such a diet was potentially too low or that such a diet was relatively uncommon in our population, which prevented the detection of an association with subsequent cognitive performance.

With respect to the underlying biological mechanisms and the potential indirect role of such a diet, in the Introduction of the article we noted that the “MedDiet constitutes a promising approach in the prevention of cognitive decline or risk of dementia due to its direct and/or indirect impact on brain aging through its nutritional constituents which may enhance neuronal plasticity, counteract inflammation and oxidative stress, and improve vascular parameters (4–7).” Because our hypotheses concerned only the direct impact of adherence to a Mediterranean diet on cognitive performance, our analyses were extensively adjusted for vascular and cardiometabolic factors including

BMI, history of diabetes, history of hypertension, and prevalent cardiovascular diseases.

Finally, we would also like to stress that our findings do not provide any sort of “compelling evidence” against adherence to a Mediterranean diet. In fact, we acknowledge that such a diet might in fact have a potential beneficial long-term impact on health.

Indeed, we mentioned in the Introduction that a high level of adherence to such a diet has been associated with improved health outcomes (8, 9). With the use of data from the SU.VI.MAX cohort, we previously reported a beneficial role of a Mediterranean diet in metabolic syndrome (including most of its components) (10) and in overweight/obesity (11). We did not observe a direct cross-time association between adherence to a Mediterranean diet at midlife and subsequent cognitive performance, which does not preclude an overall beneficial effect of such a diet on morbidity and mortality, arguing for the promotion of the Mediterranean diet in a primary prevention context.

None of the authors had any conflicts of interest related to the contents of this letter.

*Emmanuelle Kesse-Guyot
Valentina A Andreeva
Camille Lassale
Serge Hercberg
Pilar Galan*

Université Paris
13 Sorbonne Paris Cité UREN
(Nutritional Epidemiology Research Unit)
Inserm (U557) Inra (U1125)
Conservatoire National des Arts et Métiers
93017 Bobigny France
E-mail: e.kesse@uren.smbh.univ-paris13.fr

REFERENCES

1. Kesse-Guyot E, Andreeva VA, Lassale C, Ferry M, Jeandel C, Hercberg S, Galan P. Mediterranean diet and cognitive function: a French study. *Am J Clin Nutr* 2013;97:369–76.
2. Kesse-Guyot E, Amieva H, Castetbon K, Henegar A, Ferry M, Jeandel C, Hercberg S, Galan P. Adherence to nutritional recommendations and subsequent cognitive performance: findings from the prospective Supplementation with Antioxidant Vitamins and Minerals 2 (SU.VI.MAX 2) study. *Am J Clin Nutr* 2011;93:200–10.
3. Kesse-Guyot E, Andreeva VA, Jeandel C, Ferry M, Hercberg S, Galan P. A healthy dietary pattern at midlife is associated with subsequent cognitive performance. *J Nutr* 2012;142:909–15.
4. Frisardi V, Panza F, Seripa D, Imbimbo BP, Vendemiale G, Pilotto A, Solfrizzi V. Nutraceutical properties of Mediterranean diet and cognitive decline: possible underlying mechanisms. *J Alzheimers Dis* 2010;22:715–40.
5. Kamphuis PJ, Scheltens P. Can nutrients prevent or delay onset of Alzheimer's disease? *J Alzheimers Dis* 2010;20:765–75.
6. Ramesh BN, Rao TS, Prakasam A, Sambamurti K, Rao KS. Neuro-nutrition and Alzheimer's disease. *J Alzheimers Dis* 2010;19:1123–39.
7. Joseph J, Cole G, Head E, Ingram D. Nutrition, brain aging, and neurodegeneration. *J Neurosci* 2009;29:12795–801.
8. Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *Am J Clin Nutr* 2010;92:1189–96.
9. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. *BMJ* 2008;337:a1344.

10. Kesse-Guyot E, Ahluwalia N, Lassale C, Hercberg S, Fezeu L, Lairon D. Adherence to Mediterranean diet reduces the risk of metabolic syndrome: a 6-year prospective study. *Nutr Metab Cardiovasc Dis* (Epub ahead of print 25 May 2012).
11. Lassale C, Fezeu L, Andreeva VA, Hercberg S, Kengne AP, Czernichow S, Kesse-Guyot E. Association between dietary scores and 13-year weight change and obesity risk in a French prospective cohort. *Int J Obes Lond* 2012;36:1455–62.

doi: 10.3945/ajcn.113.062398.

Self-report-based estimates of energy intake offer an inadequate basis for scientific conclusions

Dear Sir:

It has been 23 y since one of us (DAS) examined data from 9 studies that compared self-reported energy intakes (EIs) with measurements of EI made by use of the doubly labeled water (DLW) method. At that time, we detected substantial biases and inaccuracies in self-reported EIs such that we concluded, “Because the greatest bias was observed in obese subjects, current methods for self-reported energy intake are not recommended for use in obesity research” (1). Despite this, yet another new publication has used self-report data and arrived at the conclusion that “after decades of increases, mean energy intake has decreased significantly since 2003–2004” (2). Although the CDC has led a historic effort to reverse the obesity epidemic, that does not justify their investigators using 40 y of self-reported EI data from the NHANES unless some reason exists to conclude that the accuracy of dietary reporting has substantially improved since the publication of those earlier caveats. Unfortunately, this is not the case. Rather, other reports would suggest that bias in reporting of energy intake may even have increased (3). Hence, the conclusions of Ford and Dietz (2) cannot be supported by the EI data they present.

Are the EI data from NHANES valid?

The average values for EI in the NHANES data set range from a low of 1972 kcal/d to a high of 2267 kcal/d. These values are not physiologically plausible. With the advent of the DLW method, it became possible to compare EI with objectively measured total energy expenditure and hence energy requirements for weight maintenance (4). The Observing Protein and Energy Nutrition study in 484 middle-aged, healthy adult subjects used the National Cancer Institute’s 5-pass 24-h recall system, a protocol almost identical to the NHANES method for collecting dietary data that has been in use for more than a decade, and found that self-reported intakes averaged 2170 kcal/d. This figure is very similar to the 2220 kcal/d reported by Ford and Dietz for that same 1999–2000 period. Biomarker total energy expenditure, however, averaged 2532 kcal/d, indicating a 14% underreporting of EI by self-report. This finding is not an outlier. Systematic underreporting of EI has been found in almost all validations of EI against objectively measured energy expenditure, thus repeatedly showing that self-reported EI is invalid (5, 6). Indeed, we can apply thermodynamic models (7, 8) to express the self-reported EI reduction of 98 kcal/d between 2001–2005 and 2007–2010 in terms of weight loss. For example, 2 separate body weight calculators (<http://bwsimulator.niddk.nih.gov/> and <http://www.pbrc.edu/research-and-faculty/calculators/sswcp/>) consistently predicted an improbable population-wide

weight loss of ~3.5 kg resulting from this self-reported reduction in EI.

Might the reporting error have increased in recent years?

Not only are self-reported EI data subject to too much systematic error (bias) to be used as a research tool to track energy balance, but we think it is plausible that the magnitude of the bias may have increased in recent years. Furthermore, it is likely that the reduction in self-reported EI does not reflect a reduction in actual EI. The degree of underreporting is positively related to BMI, at least partially under volitional control, and is likely motivated by social desirability (9). Furthermore, as has been shown, interventions that emphasize the importance of some behaviors can lead to increases in reporting error because the participants modify their reports in the desired direction independently of actual behavior change (10). In other words, we “teach” subjects the relevant socially acceptable responses (11). As such, ubiquitous media and public health messages about the importance of combating obesity (12), in which the primary focus has been on eating less, may explain the increasing downward biases in self-reported EI. It is also likely that increased underreporting of foods high in sugar and fat has played a role (3). The increased downward bias in self-reported EI may parallel the increased downward bias in self-reported weight exhibited in recent years (13).

Conclusions

In conclusion, the data analyzed by Ford and Dietz do not justify the conclusion that EI has decreased among US adults in recent years. We recognize that there is great interest in studying food intake among free-living individuals in large samples. We also recognize that in such large studies, self-report may be the only measurement tool that is practical and may well provide data in regard to dietary patterns. Although Ford and Dietz acknowledged the limitations of self-reported food intake, the common argument that it is the best available method does not make it adequate. Erroneous conclusions derived from self-reported EI may adversely affect policy decisions involving obesity.

Lord Kelvin once said, “In physical science a first essential step in the direction of learning any subject is to find principles of numerical reckoning and practicable methods for measuring some quality connected with it. I often say that when you can measure what you are speaking about and express it in numbers you know something about it” (14). More than 20 y ago when DLW data became available, the assumption that self-reports of EI could be used to generate valid conclusions came to an end. Going forward, we should accept that self-reported EI is fatally flawed and we should stop publishing inaccurate and misleading EI data. With the advent of new validated tools for estimating EI, such as thermodynamic models (7, 8), remote sensing devices (15, 16), and remote food photography (17), we should work toward applying, improving, and extending these methods for measuring EI in free-living persons rather than continuing to rely on self-report.

None of the authors has a direct financial connection, such as stock ownership, etc, to organizations that have a financial interest in the outcome or topic of this letter. Collectively, the authors have consulted for and/or received research funds from government organizations, nonprofit organizations, food and beverage companies, pharmaceutical companies, weight-loss companies, and companies that market devices for physical activity.

*Dale A Schoeller**John Foreyt*

Department of Nutritional Sciences
University of Wisconsin-Madison
Madison, WI

Baylor College of Medicine
Houston, TX

*Diana Thomas**Nikhil V Dhurandhar*

Department of Mathematical Sciences
Montclair State University
Montclair, NJ

Infection and Obesity Laboratory
Pennington Biomedical Research Center
Baton Rouge, LA

*Edward Archer**John G Kral*

Department of Exercise Science
University of South Carolina
Columbia, SC

Department of Surgery
SUNY Downstate Medical Center
New York, NY

*Steven B Heymsfield**Kevin D Hall*

Pennington Biomedical Research Center
Baton Rouge, LA

Laboratory of Biological Modeling
National Institute of Diabetes and Digestive and Kidney
Diseases
National Institutes of Health
Bethesda, MD

Steven N Blair

Arnold School of Public Health
University of South Carolina
Columbia, SC

*Barbara C Hansen**Michael I Goran*

Department of Internal Medicine
College of Medicine
University of South Florida
Tampa, FL

University of Southern California (USC) Diabetes and Obesity
Research Institute
Department of Preventive Medicine
USC Keck School of Medicine
Los Angeles, CA

*Berit Lilienthal Heitmann**James O Hill*

Institute of Preventive Medicine
Research Unit for Dietary Studies
Frederiksberg Hospital
Frederiksberg
Denmark

Anschutz Health and Wellness Center
University of Colorado
Aurora, CO

*Eric Ravussin**Richard L Atkinson*

Pennington Biomedical Research Center
Louisiana State University System
Baton Rouge, LA

Obetech Obesity Research Center
Richmond, VA

*David B Allison**Barbara E Corkey*

Obesity Research Center
Department of Medicine
Boston University School of Medicine
Boston, MA

Nutrition Obesity Research Center
University of Alabama at Birmingham
1665 University Boulevard
University of Alabama at Birmingham
Birmingham, AL 35294-0022
E-mail: dallison@uab.edu

REFERENCES

- Schoeller DA, Bandini LG, Dietz WH. Inaccuracies in self-reported intake identified by comparison with the doubly labelled water method. *Can J Physiol Pharmacol* 1990;68:941–9.
- Ford ES, Dietz WH. Trends in energy intake among adults in the United States: findings from NHANES. *Am J Clin Nutr* 2013;97:848–53.
- Heitmann BL, Lissner L, Osler M. Do we eat less fat, or just report so? *Int J Obes Relat Metab Disord* 2000;24:435–42.
- Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, Sharbaugh CO, Trabulsi J, Runswick S, Ballard-Barbash R, et al. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. *Am J Epidemiol* 2003;158:1–13.
- Trabulsi J, Schoeller DA. Evaluation of dietary assessment instruments against doubly labeled water, a biomarker of habitual energy intake. *Am J Physiol Endocrinol Metab* 2001;281:E891–9.
- Scagliusi FB, Ferriolli E, Pfrimer K, Laureano C, Cunha CS, Gualano B, Hatzlhofer Lourenco B, Lancha AH. Underreporting of energy intake in Brazilian women varies according to dietary assessment: a cross-sectional study using doubly labeled water. *J Am Diet Assoc* 2008;108:2031–40.
- Hall KD, Sacks G, Chandramohan D, Chow CC, Wang YC, Gortmaker SL, Swinburn BA. Quantification of the effect of energy imbalance on bodyweight. *Lancet* 2011;378:826–37.
- Thomas DM, Martin CK, Heymsfield S, Redman LM, Schoeller DA, Levine JA. A simple model predicting individual weight change in humans. *J Biol Dyn* 2011;5:579–99.
- Heymsfield SB, Darby PC, Muhlheim LS, Gallagher D, Wolper C, Allison DB. The calorie: myth, measurement, and reality. *Am J Clin Nutr* 1995;62(suppl):1034S–41S.
- Taber DR, Stevens J, Murray DM, Elder JP, Webber LS, Jobe JB, Lytle LA. The effect of a physical activity intervention on bias in self-reported activity. *Ann Epidemiol* 2009;19:316–22.
- Racette SB, Schoeller DA, Kushner RF, Neil KM. Exercise enhances dietary compliance during moderate energy restriction in obese women. *Am J Clin Nutr* 1995;62:345–9.
- Hilton S, Patterson C, Teyhan A. Escalating coverage of obesity in UK newspapers: the evolution and framing of the “obesity epidemic” from 1996 to 2010. *Obesity (Silver Spring)* 2012;20:1688–95.
- Shiely F, Hayes K, Perry IJ, Kelleher CC. Height and weight bias: the influence of time. *PLoS ONE* 2013;8:e54386.
- Kelvin WT. Popular lectures and addresses. Vol 1. Constitution of matter. London, United Kingdom: Macmillan, 1891.
- Sazonov ES, Makeyev O, Schuckers S, Lopez-Meyer P, Melanson EL, Neuman MR. Automatic detection of swallowing events by acoustical means for applications of monitoring of ingestive behavior. *IEEE Trans Biomed Eng* 2010;57:626–33.
- Dong Y, Hoover A, Scisco J, Muth E. A new method for measuring meal intake in humans via automated wrist motion tracking. *Appl Psychophysiol Biofeedback* 2012;37:205–15.
- Martin CK, Correa JB, Han H, Allen HR, Rood JC, Champagne CM, Gunturk BK, Bray GA. Validity of the Remote Food Photography Method (RFPM) for estimating energy and nutrient intake in near real-time. *Obesity (Silver Spring)* 2012;20:891–9.

doi: 10.3945/ajcn.113.062125.

PUFAs in sickle cell disease

Dear Sir:

Sickle cell disease, an autosomal recessive genetic disorder characterized by a specific mutation in the β -globin gene, contributes to increased incidence of vaso-occlusive crisis, stroke, and anemia (1). In addition, hypoxemia, hyperemia, and silent cerebral infarction seen in ~22% of those with sickle cell anemia may result in cognitive dysfunction (2). Because there is no direct correlation between the number of irreversible sickled cells and vaso-occlusive crisis, Daak et al (3) studied a possible beneficial action of supplementation of n–3

PUFAs, EPA (20:5n–3) and DHA (22:6n–3), in patients with homozygous sickle cell disease in a randomized, placebo-controlled, double-blind trial and noted a significant decrease in the vaso-occlusive events, severe anemia, blood transfusion requirement, and white blood cell count and overall improvement in their general condition. This clinical study was based on the previous observations that steady state sickle cell disease has abnormal red blood cell, platelet, and mononuclear cell PUFA composition in the form of elevated arachidonic acid (AA, 20:4n–6), adrenic acid (22:4n–6), and osbond acid (22:5n–6) and decreased linoleic acid (18:2n–6), EPA, and DHA (4).

In this context, it may be noted that in sickle cell anemia there could occur a deficiency of prostacyclin (PGI₂) and an excess of thromboxanes and leukotrienes and other proinflammatory and proaggregatory eicosanoids, which results in an imbalance between pro- and antiaggregatory substances, leading to adhesion of red blood cells, platelets, and mononuclear cells to the endothelium, which may lead to veno-occlusion (5–9). PGI₂, thromboxane (TX) B₂ and leukotriene (LT) B₄, and LTD₄ are derived from AA, and enhanced concentrations of AA were noted in sickle cell disease, which led to the suggestion that enhanced plasma concentrations of AA are the source of these proaggregatory substances. It is noteworthy that eicosanoids derived from EPA, although less proaggregatory, are nevertheless proaggregatory in nature. Hence, I suggest that the beneficial action of EPA and DHA observed by Daak et al (3) is unlikely to be due to the fatty acids themselves and/or formation of less proinflammatory and less proaggregatory eicosanoids but could be ascribed to the formation of lipoxins, resolvins, and protectins from various PUFAs that are potent antiinflammatory and antiaggregatory bioactive lipids.

Under normal physiologic conditions, it is likely that a balance is maintained between pro- and antiaggregatory substances. Thus, veno-occlusion seen in sickle cell anemia, at least in part, could be attributed to a deficiency of antiaggregatory and antiinflammatory substances. In this context, it is important to note that AA, EPA, and DHA form precursors to antiaggregatory and antiinflammatory bioactive lipids: lipoxins, resolvins, and protectins. I suggest that altered concentrations of AA, EPA, and DHA in sickle cell anemia leads to decreased formation of lipoxins, resolvins, and protectins. The enhanced concentrations of proaggregatory thromboxanes, leukotrienes, and other eicosanoids in sickle disease anemia reflect the absence of negative feedback regulatory control exerted by lipoxins, resolvins, and protectins on the formation of proaggregatory eicosanoids. Furthermore, PUFAs and lipoxins, resolvins, and protectins seem to have antibiotic-like actions (10, 11), which may be an added benefit to these patients, because patients with sickle cell anemia are more prone to develop infections.

In this context, it is interesting to note that forced expression of testicular receptor (TR) 2/TR4 (a pair of nuclear receptor “orphans”) in adult murine erythroid cells enhanced fetal γ -globin gene expression in transgenic mice, resulting in elevated fetal hemoglobin (HbF) synthesis, and alleviated the disease phenotype. In a “humanized” sickle cell model mouse, forced TR2/TR4 expression increased HbF abundance from 7.6% of total hemoglobin to 18.6%, accompanied by increased hematocrit from 23% to 34% and a reduction in reticulocytes from 61% to 18%, suggesting a significant reduction in hemolysis, reduced hepatosplenomegaly, and liver parenchymal necrosis indicating alleviation of pathophysiologic characteristics (12). Furthermore, reduced cluster of differentiation (CD) 36 expression with reduced foam cell formation was noted in TR4^{-/-} mice due to the ability of TR4 to induce CD36 protein and mRNA expression via transcriptional regulation. Interestingly, it was reported that TR4-mediated CD36 transactivation can be further enhanced by n–3 and n–6 PUFAs and their metabolites such as 15-hydroxyeicosatetraenoic acid (15-HETE) and 13-hydroxyocta-decaenoic acid (13-HODE) (13). These results suggest that the beneficial action of EPA/DHA

noted by Daak et al (3) could be due to enhanced formation of HbF as a result of the action of these fatty acids on TR2/TR4.

Hence, measuring plasma and urinary concentrations of lipoxins, resolvins, and protectins and HbF in patients with sickle cell anemia supplemented with EPA/DHA may prove to be interesting. As discussed above, further studies are needed to know whether administration of lipoxins, resolvins, and protectins may be beneficial in the prevention and management of sickle cell crisis, veno-occlusion, and other complications of sickle cell anemia. Furthermore, these bioactive lipids have a regulatory role in the survival, proliferation, and differentiation of stem cells (14, 15), which implies that the use of lipoxins, resolvins, and protectins may be useful in overcoming the development of anemia, partly by enhancing the generation of HbF.

The author was in receipt of Ramalingaswami Fellowship of the Department of Biotechnology, New Delhi, during the tenure of this work. The author did not declare any conflicts of interest.

Undurti N Das

UND Life Sciences
13800 Fairhill Road, No. 321
Shaker Heights, OH 44120
E-mail: undurti@hotmail.com

REFERENCES

- Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, Klug PP. Mortality in sickle cell disease: life expectancy and risk factors for early death. *N Engl J Med* 1994;330:1639–44.
- Hogan AM, Pit-ten Cate IM, Vargha-Khadem F, Prengler M, Kirkham FJ. Physiological correlates of intellectual function in children with sickle cell disease: hypoxaemia, hyperaemia and brain infarction. *Dev Sci* 2006;9:379–87.
- Daak AA, Ghebremeskel K, Hassan Z, Attallah B, Azan HB, Elbashir MI, Crawford M. Effect of omega-3 (n-3) fatty acid supplementation in patients with sickle cell anemia: randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* 2013;97:37–44.
- Glew RH, Casados JK, Huang YS, Chuang LT, VanderJagt DJ. The fatty acid composition of the serum phospholipids of children with sickle cell disease in Nigeria. *Prostaglandins Leukot Essent Fatty Acids* 2002;67:217–22.
- Stuart MJ, Sills RH. Deficiency of plasma prostacyclin or PGI2 regenerating ability in sickle cell anaemia. *Br J Haematol* 1981;48:545–50.
- Longenecker GL, Mankad V. Decreased prostacyclin levels in sickle cell disease. *Pediatrics* 1983;71:860–1.
- Setty BN, Chen D, Stuart MJ. Sickle cell vaso-occlusive crisis is associated with abnormalities in the ratio of vasoconstrictor to vasodilator prostanoids. *Pediatr Res* 1995;38:95–102.
- Ibe BO, Kurantsin-Mills J, Raj JU, Lessin LS. Plasma and urinary leukotrienes in sickle cell disease: possible role in the inflammatory process. *Eur J Clin Invest* 1994;24:57–64.
- Setty BN, Stuart MJ. Eicosanoids in sickle cell disease: potential relevance of neutrophil leukotriene B4 to disease pathophysiology. *J Lab Clin Med* 2002;139:80–9.
- Das UN. Antibiotic-like actions of essential fatty acids. *Can Med Assoc J* 1985;132:1350.
- Chiang N, Fredman G, Backhed F, Oh SF, Vickery T, Schmidt BA, Serhan CN. Infection regulates pro-resolving mediators that lower antibiotic requirements. *Nature* 2012;484:524–8.
- Campbell AD, Cui S, Shi L, Urbonya R, Mathias A, Bradley K, Bonsu KO, Douglas RR, Halford B, Schmidt L, et al. Forced TR2/TR4 expression in sickle cell disease mice confers enhanced fetal hemoglobin synthesis and alleviated disease phenotypes. *Proc Natl Acad Sci USA* 2011;108:18808–13.
- Xie S, Lee Y-F, Kim E, Chen L-M, Ni J, Fang L-Y, Liu S, Lin S-J, Abe J-I, Berk B, et al. TR4 nuclear receptor functions as a fatty acid sensor to modulate CD36 expression and foam cell formation. *Proc Natl Acad Sci USA* 2009;106:13353–8.
- Das UN. Influence of polyunsaturated fatty acids and their metabolites on stem cell Biology. *Nutrition* 2011;27:21–5.
- Das UN. Essential fatty acids and their metabolites as modulators of stem cell biology with reference to inflammation, cancer and metastasis. *Cancer Metastasis Rev* 2011;30:311–24.

doi: 10.3945/ajcn.113.061804.

Reply to UN Das

Dear Sir:

We thank Das for his interest in our article (1) and for proposing a possible mechanism for the efficacy of omega-3 (n-3) fatty acid in ameliorating vaso-occlusive crisis and hemolytic events in homozygous sickle cell patients.

The purpose of our randomized placebo-controlled trial was to investigate efficacy and safety and not to elucidate mechanisms underlying the beneficial effects of omega-3 fatty acids. Nevertheless, the points raised by Das are worth discussing because of their relevance for future mechanistic studies.

Das, after highlighting the chronic inflammatory and hypercoagulable state that characterizes sickle cell disease and the imbalances of prostacyclin (PGI₂), thromboxanes, and leukotrienes observed in patients with the disease, stated that the beneficial action of EPA and DHA in patients with the disease is “unlikely to be due to the fatty acids themselves and/or formation of less proinflammatory and less proaggregatory eicosanoids but could be ascribed to the formation of lipoxins, resolvins, and protectins from various PUFAs that are potent antiinflammatory and antiaggregatory bioactive lipids.” Although it is not justifiable to be so conclusive without any supporting evidence, we do not necessarily disagree with his assertion. Indeed, in our article, the carefully worded paragraph related to the potential mechanisms does not exclude the suggested mode of action for the efficacy. It reads, “EPA and DHA, and their respective metabolites, are known to exert a myriad of biochemical and biological effects, directly and indirectly, including through competitive inhibition of actions of AA and its metabolites. However, the synergistic effects of decreased inflammation, blood cell aggregation, adhesion and oxidative stress, and of increased vasodilation and blood flow may have played a critical role in the amelioration of vaso-occlusive and hemolytic crises in the patients.”

It is widely accepted that fetal hemoglobin is a potent modulator of hematologic and clinical features of sickle cell disease (2), and lipoxins, resolvins, and protectins have been shown to have ubiquitous function and diverse potential clinical benefits (3–5). Hence, we fully agree with Das that future studies pertaining to omega-3 fatty acids and sickle cell disease should measure the aforementioned variables.

Das states, “Further studies are needed to know whether administration of lipoxins, resolvins, and protectins may be beneficial in the prevention and management of sickle cell crisis, vaso-occlusion, and other complications of sickle cell anemia.” This is a very interesting idea that is worth testing, perhaps initially in an animal model of the disease. Sickle disease is a multifactorial condition with a diverse and wide spectrum of biochemical, hematologic, and clinical features such as vaso-occlusion (6), hemolysis (7), oxidative stress (8), chronic inflammation (9), and blood cell membrane defects (10). Therefore, a single multifunctional or combination therapy is necessary to help ameliorate the varied abnormalities of the disease. Indeed, the reason we hypothesized that omega-3 fatty acids (EPA and DHA) might be an effective treatment of sickle cell disease is because of their role in receptor function, signaling, membrane

fluidity, and oxidative protection and as precursors of bioactive compounds. Because bioactive lipids are synthesized from membrane fatty acids, a proportion of the supplemented EPA and DHA, after incorporation, would have been converted to their respective metabolites. Regardless, the reductionist reasoning of attributing the observed beneficial effects of EPA and DHA to the synthesized lipoxins, resolvins, and protectins seems to disregard the possible contributions of the other vital biological functions of omega-3 fatty acids in the mitigation of vaso-occlusive and hemolytic crises in the patients. In our opinion, bioactive lipids might be effective in alleviating inflammation in sickle cell patients but they are unlikely to constitute an effective substitution of omega-3 fatty acids in the management of sickle cell disease.

The authors did not have any conflicts of interest.

*Ahmed A Daak
Kebreab Ghebremeskel*

Faculty of Life Sciences and Computing
London Metropolitan University
166-220 Holloway Road
London N7 8DB
United Kingdom
E-mail: k.ghebremeskel@londonmet.ac.uk

*Zahir Hassan
Bakhita Attallah
Haj H Azan
Mustafa I Elbashir*

University of Khartoum
Khartoum
Sudan

Michael Crawford

Faculty of Life Sciences and Computing
London Metropolitan University
London
United Kingdom

REFERENCES

1. Daak AA, Ghebremeskel K, Hassan Z, Attallah B, Azan HH, Elbashir MI, Crawford M. Effect of omega-3 (n-3) fatty acid supplementation in patients with sickle cell anemia: randomized, double-blind, placebo-controlled trial. *Am J Clin Nutr* 2013;97:37-44.
2. Akinsheye I, Alsultan A, Solovieff N, Ngo D, Baldwin CT, Sebastiani P, Chu DH, Steinberg MH. Fetal hemoglobin in sickle cell anemia. *Blood* 2011;118:19-27.
3. Serhan CN, Petasis NA. Resolvins and protectins in inflammation resolution. *Chem Rev* 2011;111:5922-43.
4. Kantarci A, Van Dyke TE. Lipoxins in chronic inflammation. *Crit Rev Oral Biol Med* 2003;14:4-12.
5. Spite M, Serhan CN. Novel lipid mediators promote resolution of inflammation. *Circulation* 2010;107:1170-84.
6. Kaul DK, Nagel RL. Sickle cell vasoocclusion: many issues and some answers. *Experientia*. 1993;49:5-15.
7. Kato GJ, Gladwin MT, Steinberg MH. Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. *Blood Rev* 2007;21:37-47.
8. Wood KC, Granger DN. Sickle cell disease: role of reactive oxygen and nitrogen metabolites. *Clin Exp Pharmacol Physiol* 2007;34:926-32.
9. Hebbel RP, Osarogiagbon R, Kaul D. The endothelial biology of sickle cell disease: inflammation and a chronic vasculopathy. *Microcirculation* 2004;11:129-51.
10. Kuypers FA. Membrane lipid alterations in hemoglobinopathies. *Hematology Am Soc Hematol Educ Program* 2007;2007:68-73.

doi: 10.3945/ajcn.113.061952.