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A Meta-Analysis Shows That Docosahexaenoic Acid from Algal Oil Reduces Serum Triglycerides and Increases HDL-Cholesterol and LDL-Cholesterol in Persons without Coronary Heart Disease[1–3]

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Abstract

Certain algae contain the (n-3) fatty acid DHA, yet the relation between algal oil supplementation and cardiovascular disease risk factors has not been systematically examined. Our objective was to examine the relation between algal oil supplementation and cardiovascular disease risk factors. We conducted a systematic review of randomized controlled trials published between 1996 and 2011 examining the relation between algal oil supplementation and cardiovascular disease risk factors and performed a meta-analysis of the association between algal oil DHA supplementation and changes in the concentrations of TG, LDL-cholesterol (LDL-C), and HDL-cholesterol (HDL-C). We identified 11 randomized controlled trials with 485 healthy participants that evaluated the relation between algal oil DHA supplementation and TG, LDL-C, and HDL-C. The median dose of algal DHA was 1.68 g/d. The pooled estimate for the change in TG concentration was −0.20 mmol/L (95% CI: −0.27 to −0.14), 0.23 mmol/L (95% CI: 0.16–0.30) for LDL-C, and 0.07 mmol/L (95% CI: 0.05–0.10) for HDL-C. DHA supplementation from algal oil, a marine source of (n-3) fatty acids not extracted from fish, may reduce serum TG and increase HDL-C and LDL-C in persons without coronary heart disease. J. Nutr. 142: 99–104, 2012.

Introduction

Consumption of fatty fish high in EPA and DHA is recommended for healthy persons and those with high blood pressure, elevated TG, and CHD[8] (1–4). There may also be benefit for persons with heart failure (5). Consumption of wild marine fish is severely limited, however, because most ocean fish populations are exploited and unable to yield sustainable catches at current levels (6–8). Aquaculture, or fish farming, provides an alternative or perhaps additional source of fish for consumption, yet the practice has been associated with damage to and pollution of sensitive ecologic areas (8–10).

In the wild, fish accumulate EPA and DHA by consumption of marine algae (11,12). Recently, algae have been cultivated to preferentially produce DHA and their oil has been extracted to make supplements (13,14). Yet although narrative reviews have been conducted on the relation between algal oil supplements and CHD risk factors, such as serum lipids (15,16), there have not yet been systematic examinations. Given the clinical, public health, and ecologic importance of understanding algal oil supplementation, we performed a systematic review and meta-analysis of published, randomized, controlled clinical trials that evaluated and quantified the association between algal oil and cardiovascular disease risk factors.

Materials and Methods

We observed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for this systematic review and meta-analysis (17).

Search strategy. We searched for all randomized controlled trials that examined the association between algae-derived EPA or algae-derived DHA and serum lipoproteins or cardiovascular disease. We searched the following online databases through May 12, 2011: MEDLINE, EMBASE, CABI (CAB abstracts), Cochrane Central Registry of Controlled Trials, www.clinicalstudyresults.org (a Web-based database of results from trials of U.S.-marketed pharmaceuticals), and www.clinicaltrials.gov (a registry of federally and privately supported clinical trials in the U.S. and around the world) (The search strategy is available in the online material). There were no language restrictions in our search.

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[3] Supplemental Tables 1–5 and Supplemental Methods are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[8] Abbreviations used: CHD, coronary heart disease; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.
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We excluded studies of animals and children and of persons with diseases that may affect absorption or metabolism of algal oil (e.g., patients with cystic fibrosis).

Study selection. Our search spanned all published studies prior to May, 2011. In our first round of searching in May, 2010, we initially located 756 articles: 185 articles from MEDLINE, 201 from EMBASE, 317 from CABI, and 53 from Cochrane Central Registry of Controlled Trials. We did not identify any relevant completed trials from www.clinicaltrials.gov. An additional MEDLINE search for articles written by select authors and a review of their bibliographies identified an additional 9 articles. A total of 736 articles were excluded after reviewing the title or abstract. Full texts of the remaining 29 were selected for secondary review. In May, 2011, we conducted an updated search for newly published articles and identified one additional article. We did not identify any studies that included cardiovascular disease outcomes. We identified studies that evaluated algal oil supplementation on thrombosis (18,19), inflammatory markers (19–22), blood pressure (19,21–23), plasma lipids (FFA) (18–20,22,24–31), and erythrocyte membrane lipids (19,20,24,26,27,30–32), but given the potential for unstable estimates from a limited amount of data due to small number of participants in these studies, we did not include these endpoints in our meta-analysis and instead focused on the relation between algal oil supplementation and serum lipids. We included 11 unique studies in the final analysis (18,19,22,23,26,28,29,33–36).

Data extraction and quality assessment. For each of the final 11 studies, we reviewed and extracted the following data using a piloted form: first author and study year, number of study participants, mean age of study participants, sex distribution of participants, study design (randomization, blinding, presence of placebo, parallel or crossover design), dose of EPA or DHA in the algal oil supplement as well as other fatty acids, content of placebo, duration of intervention, whether the study was funded by industry, and whether the algal oil was gifted to the investigators by industry and, if so, whether there was independent assessment of the supplement content. We examined whether each study used fasting blood samples for measuring lipid concentration, whether the LDL-C concentration was derived with the Friedewald equation (37), and if LDL particle size was estimated. The principal study outcomes extracted were the mean and SEM of the treatment effect of algal oil supplementation on TG, HDL-C, and LDL-C. For studies that did not directly report the treatment effect, we calculated it from the difference in the within-group estimates of change in lipid concentration (18,22,23,29,33,34,36) or from the difference in the final mean concentration of lipid concentrations (28). For studies that did not directly report the variance, we calculated it from data provided (**Supplemental Methods**). One study reported two intervention groups with one control group (29); for our analysis, we collapsed the two intervention groups into one group and calculated an average intervention dose and average treatment effect to avoid statistical duplication in the meta-analysis. All algal oil supplements used in the analyzed studies contained either no EPA (18,22,23,29,33,35,36) or a negligible amount of EPA (19,26,28,34) (e.g., 1/20 the amount of DHA/d or <0.005 g/d). To convert mg/dL of TG to mmol/L, we divided by 89; to convert mg/dL of HDL-C or LDL-C to mmol/L of HDL-C or LDL-C, we divided by 39 (38).

The quality of each study, defined as the confidence that its design, conduct, analysis, and presentation limited bias in its results, was assessed by a validated quality scoring system (39). The scoring system awarded points to each study based on: *1*) the presence of randomization; *2*) appropriate method of randomization (e.g., using random numbers); *3*) presence of double-blinding; *4*) appropriate method of double-blinding (e.g., using supplements with similar appearance); and *5*) description of withdrawals and dropouts. The lowest possible quality score was 0 and the maximum was 5.

Statistical analysis. To derive a summary (pooled) estimate of the association between algal oil DHA supplementation (g/d) and serum TG, LDL-C, and HDL-C concentrations (mmol/L), we performed a fixed-effects meta-analysis of the treatment effect, which was equal to the weighted mean difference in serum lipid concentration between intervention and control groups (*metan* procedure, STATA 11, StataCorp). The fixed-effects model assumes that the true effect of treatment is the same in each study and that differences between study results are due to chance (40). Study weights were determined by the inverse variance method so that the weight given to a particular study was the reciprocal of the squared SEE estimate. We derived an estimate of the pooled percent change in TG, LDL-C, and HDL-C due to algal oil supplementation by dividing the weighted pooled absolute change by the mean baseline lipid concentration (mean baseline concentration equals the sum of mean baseline concentration in each study multiplied by its fixed-effects weight). Magnitude of between-study heterogeneity was examined by determination of the Q statistic and I-squared statistic (41). Forest plots were used to visually graph the association between algal oil DHA supplementation and changes in absolute concentrations of serum TG, HDL-C, and LDL-C.

In sensitivity analyses to evaluate for potential dose-dependent effects, we fit three separate fixed-effects meta-regression models in which the outcomes were continuous variables for the treatment effect on TG, LDL-C, or HDL-C and the independent variable was mean algal DHA dose (g/d) (*vwls* procedure, STATA 11, StataCorp). In these meta-regression dose-effect models, the coefficient for the algal oil variable is an estimate of the dose-response relation between algal oil and change in serum lipid concentration.

Potential for publication bias was assessed using the Begg rank correlation method and Egger weighted regression test (40) for serum TG, LDL-C, and HDL-C and by inspecting three funnel plots of treatment effect compared to SE for TG, LDL-C, and HDL-C. Trim-and-fill techniques were used in sensitivity analyses to correct for potential publication bias. $P < 0.05$ was considered significant.

Results

We identified 11 randomized controlled trials, including a total of 485 participants, dating from 1996 to 2011 (18,19,22,23,26,28,29,33–36) (**Supplemental Table 1**). The mean age of study participants ranged from 24 to 59 y. Most studies were 6 wk in duration and either did not independently assess algal oil concentration or did not report such an assessment (6 of 11 studies). Every study either received the algal oil as a gift from industry or the study itself was funded by industry. All studies used fasting blood samples for lipid measurements and the majority used the Freidewald equation to estimate LDL-C. All but one of the studies reported that the algal oil contained a mixture of SFA and PUFA in addition to DHA, although the overall mean DHA fatty acid content was 41% by weight (**Supplemental Table 2**). Studies were of good quality (9 of 11 scored 4 of 5).

The median dose of algal DHA in the analysis of serum lipids was 1.68 g/d. The pooled estimate for the change in TG concentration was −0.20 mmol/L (95% CI: −0.27 to −0.14) (**Fig. 1 and Supplemental Table 3**), for LDL-C it was 0.23 mmol/L (95% CI: 0.16–0.30) (**Fig. 2 and Supplemental Table 4**), and for HDL-C it was 0.07 mmol/L (95% CI: 0.05–0.10) (**Fig. 3 and Supplemental Table 5**). A 0.20-mmol/L absolute reduction in TG corresponded to a 15% (95% CI: 10–20%) decrease, a 0.23-mmol/L increase in LDL-C corresponded to an 8% (95% CI: 6–10%) increase, and a 0.07-mmol/L increase in HDL-C corresponded to a 5% (95% CI: 4–7%) increase.

The greatest decrease in TG and greatest increase in LDL-C appeared in the two studies that included participants with elevated serum lipid concentrations at baseline (23,29). The two studies that showed a decrease in LDL-C included vegetarian participants (18,35), although the CI were wide, and a third study with vegetarian participants (34) observed a significant rise in LDL-C, similar to other studies. One study that reported using direct methods for estimating LDL-C, rather than the Freidewald equation, showed a decrease in LDL-C (35), whereas

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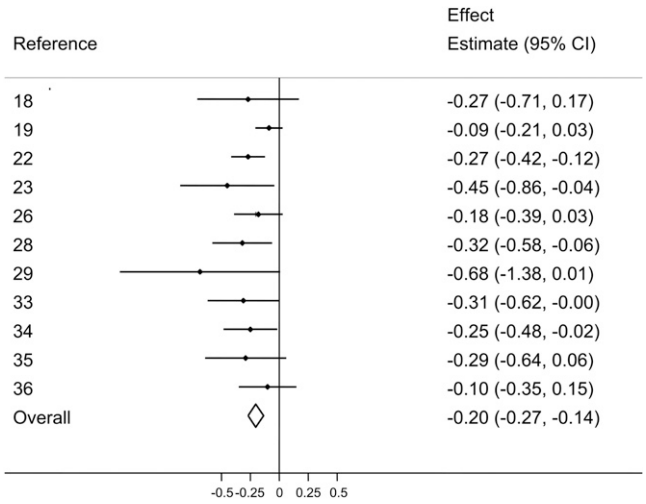

FIGURE 1 Change in serum TG (mmol/L) associated with algal DHA supplementation (g/d). Weighted mean difference in absolute change estimated; median dose of algal oil = 1.68 g/d; I-squared (variation in effect size attributable to heterogeneity) = 0.6%; Q statistic *P* value = 0.43; *n* = 11.

another that used the direct method showed no effect (33). Limiting the study to the seven studies that reported using the Friedewald equation (18,19,22,23,28,29,34), the effect estimate of change in LDL-C was 0.29 (95% CI: 0.20–0.38) mmol/L LDL-C. One study reported using "standard" methods for estimating LDL-C (36) and one did not report its method (26). The one study that showed a higher increase in HDL-C compared to other studies had young study participants, was of longer duration than most studies, and was single-blinded (33).

There was little evidence of between-study heterogeneity by Q statistic and I-squared statistic; of the total variation in each weighted mean difference model, the amount of between-study variation was 0.6% when evaluating the association between algal DHA and TG (Q statistic *P* value = 0.43), 41% for algal oil and LDL-C (Q statistic *P* value = 0.08), and 31% for algal DHA and HDL-C (Q statistic *P* value = 0.15).

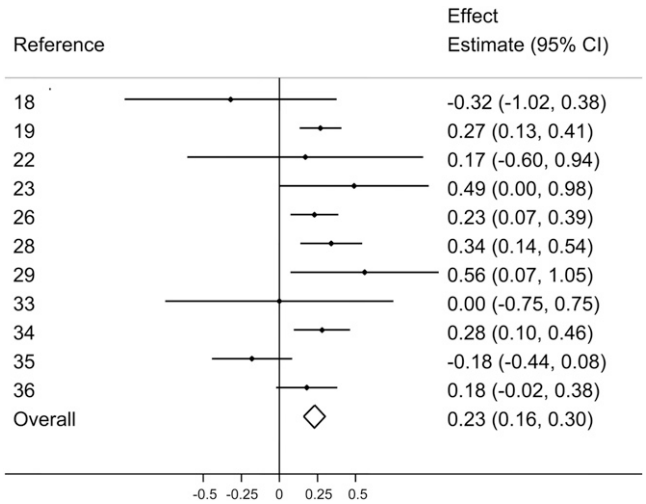

FIGURE 2 Change in serum LDL-C (mmol/L) associated with algal DHA supplementation (g/d). Weighted mean difference in absolute change estimated; median dose of algal oil = 1.68 g/d; I-squared (variation in effect size attributable to heterogeneity) = 40.8%; Q statistic *P* value = 0.08; *n* = 11. LDL-C, LDL-cholesterol.

In the meta-regression, we did not observe a significant dose response between algal DHA and TG, LDL-C, or HDL-C concentration: a 1-g increase per day in the dose of algal DHA was associated with a 0.06-mmol/L decrease in TG (95% CI: −0.19, 0.07), a 0.04-mmol/L reduction in LDL-C (95% CI: −0.28, 0.19), and a 0.02-mmol/L reduction in HDL-C (95% CI: −0.07, 0.04). All studies but one had doses of algal oil DHA <3 g/d. The magnitude of TG reduction was relatively constant between 0.7 and 2.8 g/d of algal DHA (**Fig. 4**). In the one study that gave 3 g/d of algal oil DHA, there was a further reduction in TG concentration (23).

The three funnel plots were symmetrical, suggesting that effect estimates were not associated with SE or study size. Of the three Begg rank correlation tests and three Egger tests, five were not significant and only the Egger test with serum TG was significant (*P* value 0.01), suggesting an overall small potential for publication bias or small-study effects. Trim-and-fill correction analysis with serum TG showed the main results remained significant and robust.

Discussion

To our knowledge, this is the first systematic review and meta-analysis of algae-derived DHA supplementation and serum lipids. We observed a significant decrease in TG and significant increases in both HDL-C and LDL-C. The 0.20-mmol/L (95% CI: −0.27 to −0.14) reduction in TG we observed with the 1.68-g/d dose of algal DHA is on the same order of magnitude observed with fish oil supplementation in a recent meta-analyses: 3.25 g of fish oil (including 1.90 g EPA and 1.35 g DHA) was associated with a 0.34-mmol/L (95% CI: −0.41 to −0.27) reduction in TG (42). The increase in HDL-C was also similar to that with fish oil (0.01 mmol/L; 95% CI: 0.00–0.02); the increase in LDL-C was higher (0.06 mmol/L; 95% CI: 0.03–0.09) (42).

Current AHA dietary recommendations for persons without CHD are to eat 2 servings/wk of fish (preferably oily) to reach ~0.25 g/d of the (n-3) fatty acids EPA and DHA and to reduce the risk of sudden death and death from CHD (3,43,44). The NIH has suggested 0.65 g/d of EPA and DHA and a minimum of 0.3 g/d of DHA during pregnancy and lactation (45), whereas the Institute of Medicine makes no specific recommendations for EPA and DHA intake (46). For persons with CHD, the AHA recommends 1 g/d of EPA and DHA, preferably from fish, but supplements can be considered in consultation with a physician. For patients with high TG, the recommendation is 2–4 g/d of EPA and DHA provided as capsules and under the supervision of a physician (3).

Recent data from the NHANES in 2005–2006 shows that U.S. citizens consume on average 0.05 g/d of EPA and 0.10 g/d of DHA (47), far less than that recommended either for healthy persons or individuals with high TG or CHD. Yet consumption of more fish, or extraction of their oil for supplements, may not be possible given the limitations of marine fishing. Stocks of pacific salmon, tuna, herring, mackerel, and anchovies are already fully exploited (the ocean fishery is operating at or near optimal yield with no room for further expansion) or over-exploited (there is no room for further expansion and there is a high risk for depletion/collapse of the fish stock) (6,48,49) and Atlantic salmon stocks are also fully exploited to depleted (48). Although aquaculture provides an alternative to marine fishing, there are concerns with its ecologic impact (8–10).

Fish naturally accumulate EPA and DHA by consumption of marine algae (11,12), which are a diverse group of uni- and multi-cellular photosynthetic organisms. In contrast to algae, such as seaweed, microscopic algae have been commercially

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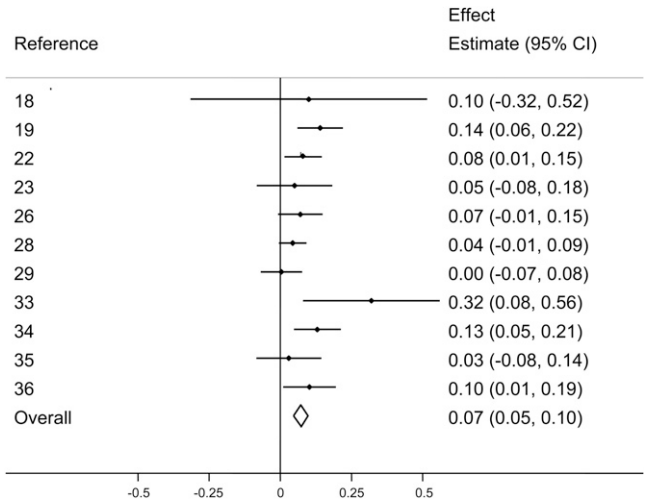

FIGURE 3 Change in serum HDL-C (mmol/L) associated with algal DHA supplementation (g/d). Weighted mean difference in absolute change estimated; median dose of algal oil = 1.68 g/d; I-squared (variation in effect size attributable to heterogeneity) = 31.4%; Q statistic *P* value = 0.15; *n* = 11. HDL-C, HDL-cholesterol.

cultivated in open ponds and closed photo-bioreactors where they preferentially produce one type of PUFA (14). Algae currently being bred in this algaculture to create DHA-rich algal oil include *Crypthecodinium cohnii*, *Schizochytrium* species, and *Ulkenia* species. Food products already fortified with algal oil include infant formula, olive and canola oils, and soy milk (50).

Purified ethyl ester DHA supplementation has been shown to lower TG and mildly elevate LDL-C and HDL-C (51,52). The 8% increase in LDL-C and 5% increase in HDL-C we observed with algal oil is similar to that observed with 4 g/d of purified DHA, the latter which was also associated with a transition to larger, possibly less-atherogenic LDL particles (52). DHA is thought to decrease TG levels by reducing hepatic VLDL synthesis (53). Reduced synthesis in turn leads to reduced secretion and smaller VLDL particles, which are more readily converted to LDL than are large VLDL particles and which also compete with LDL for uptake by LDL receptors (52). Both of these actions lead to higher serum LDL-C. The mechanism by which DHA increases HDL-C is not known but may be through decreased lipid transfer protein activity (54).

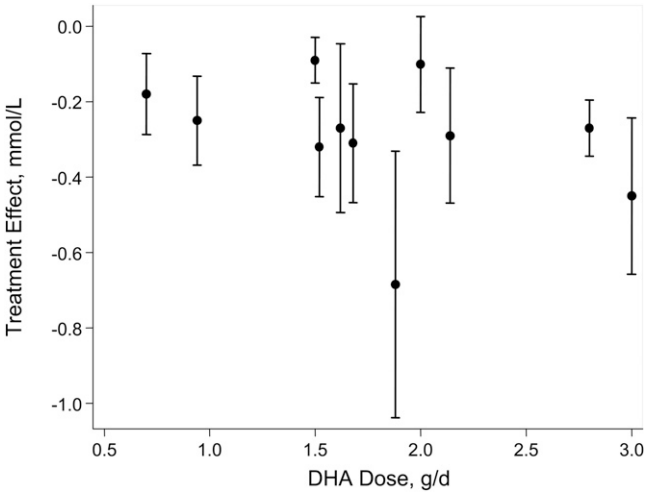

FIGURE 4 Dose of algal DHA supplementation in relation to the change in serum TG (mmol/L). Mean treatment effect and 95% CI given; *n* = 11.

All but one of the placebo arms in the trials we examined contained corn, soy, or olive oil, each of which is rich in palmitic, oleic, and linoleic acids. Soy oil also has a small amount of α-linolenic acid. Most of the algal oil supplements contained palmitic and oleic acid, though in different amounts than the placebos. The DHA content of the supplements could explain our findings; however, it is also likely that the saturated fat content of the algal oil contributed to study participant lipid concentrations different than that those expected from purified DHA. Of the six studies that measured LDL particle size in relation to algal oil supplementation, five reported an increase in particle size and one reported no change; because particle size, in addition to lipoprotein cholesterol content, may help predict atherogenic risk, the net effect of algal DHA supplementation on serum lipoproteins and lipids may be beneficial despite the increase in LDL-C (55). We also note that two earlier meta-analyses of fish oil supplementation reported increase in LDL similar to those we observed in this study with algal oil (56,57). The effect of algal oil supplementation on overall cardiovascular risk must also consider its effect on other cardiovascular disease risk factors, such as blood pressure, platelet aggregation, and inflammation.

We did not identify any reports on algal oil contamination but, according to the U.S. Dietary Supplement and Health Education Act of 1994, the FDA cannot vet supplement content before a supplement is sold. Some fish oil supplements have been found to be contaminated with persistent organic pollutants (58,59). High-dose algal oil supplementation, as with fish oil supplementation, should be done under a physician's supervision to monitor for side effects, adverse reactions, and interactions with other medications. To approximate 200 mg/d of DHA, algal oil supplementation at present may cost three times as much as fish oil supplementation (60,61).

The strengths of our analysis are that we made extensive efforts to locate all published and unpublished (i.e., abstract only) work without limitation to language, country, date, or funding. Study designs were of good quality and with an average duration of 6 wk, there was adequate time to see changes in measured outcomes. The chief limitations are that the studies were few and small, all were funded or gifted by industry, and most did not independently verify the algal oil supplements. We were also only able to assess the relation between algal oil supplementation and serum lipids. Because one of the recognized benefits of fish oil supplementation is antiarrhythmic with a reduction in sudden death, our ability to compare algal oil supplementation to fish oil supplementation is limited. Moreover, dose-response results from the meta-regression should be interpreted cautiously, because the highest dose came from a study population with high baseline TG. Thus, residual between-study confounding is a potential limitation of our dose-response analysis. We included studies that used both the indirect and direct method of estimating LDL-C because the correlation between the two methods has been reported to be between 0.94 and 0.99 (37). However, in our analysis, the different methods may have been a source of between-study heterogeneity and this possibility should be explored further. Future investigations should also compare different doses of algal oil in the same population and compare algal oil to fish oil with cardiovascular outcomes.

In conclusion, DHA supplementation from algal oil, a marine source of (n-3) fatty acids not extracted from fish, may reduce serum TG and increase LDL-C and HDL-C in persons without CHD.

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Acknowledgments

A.M.B. and E.B.R. designed and conducted the research; A.M.B., E.L.D., W.C.W., and E.B.R. analyzed data; A.M.B. wrote the paper; and E.B.R. had primary responsibility for final content. All authors read and approved the final manuscript.

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