Suppressive effect of a selective increase in plasma linoleic acid concentration and intravascular lipolysis on peripheral T cell activation

Dear Sir:

With interest we read in the Journal the findings of a recent study by Larbi et al (1), in which the authors reported on the suppressive effects on T cell activation and function, together with changes in the membrane properties of these cells, that are brought about by the intravenous administration of a soybean oil–based lipid emulsion (Intralipid 20%; Baxter, Mississauga, Canada). The authors concluded that these results should have serious implications for nutritional therapy in patients at high risk of septic complications.

We have several concerns with regard to the latter statement. First, the investigation by Larbi et al was performed in healthy subjects, and, therefore, their results cannot be extrapolated to patients with an inflammatory condition. Second, several reviews focused on immune modulation by parenteral lipids in general (2) and by soybean oil–based emulsions in particular (3) concluded that no clear picture exists, because of the very disparate outcomes of the studies reviewed. For instance, our group has consistently found that Intralipid, an emulsion that is rich in long-chain triacylglycerols, does not affect human neutrophil and mononuclear cell functions, including oxygen radical production, adhesion, chemotaxis, pathogen killing, and cytokine production (4–8). As in the study by Larbi et al, these observations were made in cells obtained from healthy volunteers and in studies with both in vitro and in vivo designs. It is important that, in our hands, Intralipid did not alter membrane fluidity, as measured by the same technique that Larbi et al used, or signal transduction of leukocytes (9, 10). In contrast, the administration of an emulsion containing medium-chain triacylglycerols in these investigations resulted in cell activation, altered cytokine responses, increased membrane fluidity, and attenuation of calcium signaling in neutrophils and mononuclear cells (4–10). In our opinion, these conflicting outcomes show that, at present, we do not fully comprehend the nature of the interaction of immune cells and parenteral lipids and the circumstances influencing that interaction.

The author had no personal or financial conflict of interest with respect to the study of Larbi et al or the report on that study.

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Reply to GJ Wanten

Dear Sir:

We read with great interest the comments of GJ Wanten concerning our recent report (1) of a reduction in circulating T cell activation and proliferation during enhanced intravascular lipolysis with Intralipid and heparin that resulted in a selective increase in plasma linoleate concentrations in healthy humans. We agree with Wanten that our results in healthy subjects cannot be directly extrapolated to the usual intensive-care-unit patients who have severe inflammatory conditions often associated with a severe catabolic state—a state that in itself may alter the immune response. As mentioned by Wanten, the data from the literature are conflicting with respect to the untoward effects of intravenous lipid emulsions on the immune function in humans. Although early studies did not find evidence of an immunosuppressive effect of total parenteral nutrition with Intralipid or other lipid emulsions in the critical or postoperative care setting (2–5), more recent randomized controlled trials found that the administration of an intravenous lipid emulsion with total parenteral nutrition was associated with less T cell proliferation, a higher rate of infection, more prolonged need for mechanical ventilation, and more prolonged stays in the intensive care unit or in the hospital (6, 7). Our findings (1) also confirmed those of several previous

Am J Clin Nutr 2006;83:918–22. Printed in USA.

studies that showed a reduced reactivity of human peripheral lymphocytes after either in vitro or in vivo exposure to Intralipid or another lipid emulsion composed of long-chain fatty acids (8–10). Thus, we believe that evidence of a role for long-chain fatty acids in the modulation of T cell function is accumulating. Whether this effect and the proposed mechanisms we showed in our study are relevant in pathophysiological states in humans clearly needs more investigation.

Our report did not address the potential role of a selective elevation of plasma linoleate concentrations on the function of circulating monocytes and neutrophils. Neutrophils and monocytes may behave very differently in response to fatty acids than do T lymphocytes. Thus, we agree with Wanten that the effect of long-chain fatty acids shown in one class of white blood cells cannot be extrapolated to other such classes and that more studies are needed to integrate the multiple potential effects of long-chain fatty acids on the immune system in healthy and pathophysiologic states in humans.

The authors had no conflict of interest.

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Effect of nutritional manipulation on brain function: implications for future research

Dear Sir:

I am writing about the recently published review by McCann and Ames (1). This review addresses the behavioral effects of n−3 fatty acids in humans and animals. The authors are to be commended on their thoroughness in researching the extensive literature on this topic and in invoking the necessary methodologic considerations when evaluating the outcomes of studies conducted in both animals and humans. In their discussion of the various behavioral studies included in this review, they raise an important issue—that different neural systems might be affected differently by nutritional manipulations, and, thus, some outcomes might be more sensitive indicators than others with respect to the role of a particular nutrient. From my perspective as a behavioral scientist who works with rodents, I would like to expand on this observation and explore the implications for future research.

Although the terms *cognitive* and *behavioral* are often used freely in relation to nutritional interventions, what is meant by these terms is not always defined precisely. Thus, whereas both the human and animal literature encompass a diverse array of behavioral outcomes, there is often little discussion of exactly which specific psychological construct is being measured by each test and how this relates to overall cognitive function. This is despite an accumulation of evidence in both humans and animals during the past 15–20 y that supports the hypothesis that the brain comprises multiple memory systems, each anatomically and biochemically distinct and each processing information in a specialized way (2). On the basis of behavioral dissociations in studies using lesions in rats, 3 independent systems have been identified: *1*) flexible use of knowledge involving the hippocampus, *2*) habitual responses (reinforced stimulus-response associations) involving the dorsal striatum, and *3*) emotional responses (Pavlovian stimulus—affect conditioning) involving the amygdala. In most situations, these systems operate in parallel, and, depending on the circumstances, interact either competitively or cooperatively. For example, depending on the protocol, different types of learning, either cognitive or stimulus-response, can occur in the place version of the Morris water maze. If the start position is varied over trials, the animal is required to use spatial information flexibly, whereas if the start position remains the same, the animal can use the response system, eg, swim 45 degrees left. What is important to note, however, is that when one system is damaged, performance will be impaired on tasks associated with the function of that particular system, but very often enhanced on the tasks associated with the other. For example, animals with hippocampal damage often perform better than do control animals on stimulus-response type learning tasks. Thus, it is entirely possible for an intervention that improves one type of memory to have deleterious effects on another type of memory or to have effects on other measures, such as arousal, that affect task performance.

In summary, the point being made herein is that broad terms such as *cognition* need to be carefully operationalized and then tested by

using a comprehensive approach based on a broad battery of valid and reliable neuropsychological tests before conclusions can be drawn as to which of the myriad aspects of brain function are affected by a particular nutritional manipulation. Only then will we be in a position to make fully informed population-based recommendations with respect to dietary interventions.

The author had no conflict of interest in relation to this letter.

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Reply to P Wainwright

Dear Sir:

We thank Wainwright for her kind words about our review on the n−3 long-chain polyunsaturated fatty acid (LCPUFA) docosahexaenoic acid (DHA) (1). Our review was the first in a series of articles intended for nonspecialists that summarize evidence for causal relations between micronutrient deficiencies during development and brain function. A review on choline will appear in early 2006 (2).

Wainwright raises an important point about the need for scientific precision in assigning results of behavioral tests to specific cognitive functions. We concur with most of her comments and would like to add some additional observations regarding the relevance of results obtained in these tests to public health decision making. Wainwright concludes her letter with the following 2 sentences: "In summary, the point being made herein is that broad terms such as *cognition* need to be carefully operationalized and then tested by using a comprehensive approach based on a broad battery of valid and reliable neuropsychological tests before conclusions can be drawn as to which of the myriad aspects of brain function are affected by a particular nutritional manipulation. Only then will we be in a position to make fully informed population-based recommendations with respect to dietary interventions."

Regarding whether the addition of DHA (in combination with AA) to infant formula should be recommended, we believe that the level of precision required is far less than that implied by Wainwright. As many others have noted, and as we summarized in our review (1), several scientific observations regarding DHA and human infants are well established:

1) DHA is present in relatively large amounts in the human brain and retina but not in most other tissues.

2) DHA is naturally present in breast milk, accretes in the brain during perinatal growth, and is maternally supplied.

3) Breastfed infants have significantly higher brain concentrations of DHA than do formula-fed infants.

4) Preterm infants are born with significantly less DHA in their brains than are term infants because significant accretion occurs in the last trimester of pregnancy.

These observations have been clearly established. In addition, DHA-containing formula supplements were granted Generally Recognized As Safe (GRAS) status by the Food and Drug Administration in 2002, are recommended for use by regulatory and advisory bodies in other countries, are widely used in Europe and Asia, and are increasingly present on grocery store shelves in the United States. Despite these facts, organizations such as the American Academy of Pediatrics (AAP), the primary source of pediatric medical practice guidelines and policy in the United States, and UpToDate.com, a widely used medical advice service for doctors, have not supported the addition of LCPUFAs to infant formula (3, 4). The AAP states that they have "no official position" on supplementation, and the most recent advice from UpToDate.com concludes that "additional studies will be needed to prove benefits of LCPUFA as well as provide guidelines for supplementation."

Our reading of the statements from both of these organizations suggests that a significant reason for their hesitation in recommending the addition of DHA to infant formula is the lack of proof of efficacy as measured by randomized controlled trials (RCTs) that compare performance on standardized tests, such as the Bayley Scales of Infant Development, of LCPUFA-supplemented or -unsupplemented formula-fed children. However, as we point out in our review (1), the mixed, but substantially negative, findings of these RCTs are not particularly surprising. Many studies reproducibly demonstrate poorer performance in cognitive and behavioral tests of rodent and primate offspring whose brains have been depleted of DHA by up to 80% of normal concentrations. However, though clearly significant, performance deficits are modest. In our opinion, these observations strongly suggest that smaller differences in brain concentrations of DHA, such as almost certainly occur between infants fed supplemented and unsupplemented formulas, may result in more subtle but still significant effects on brain function that are difficult to detect with the use of current methods. Therefore, we do not believe that the scientific evidence taken as a whole justifies the need to demonstrate performance differences in RCTs before a judgment can be made about whether to recommend the addition of LCPUFAs to infant formula.

If safety is not an issue, and it seems that there is now considerable evidence that toxicity is not a significant concern (5), why should the addition of LCPUFAs to infant formula not be encouraged? Surely DHA has an important function in the developing brain or there would not be so much of it there. This is common sense. Why should infants be deprived of this substance during a critical period of brain development because of a lack of scientific certainty in establishing a causal link to poor performance on cognitive and behavioral tests in RCTs, which are expected to have limited sensitivity?

Important scientific questions regarding the function of DHA in the brain clearly need to be resolved, and there is a need to develop more sensitive and more precise measures of effects on different neural systems, as Wainwright points out. However, this level of scientific precision may take many years to achieve. On the basis of our review of the literature (1), and as we point out in this letter, the currently available scientific evidence (taken as a whole) strongly

suggests that recommending the addition of LCPUFAs to infant formula need not wait for demonstrations of efficacy in RCTs, regardless of the precision of the performance tests used. Thus, we encourage the AAP to consider reevaluating their position on the addition of LCPUFAs to infant formula.

Neither author had a conflict of interest.

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Is the whole-diet approach better than a low-fat diet in cardiovascular risk reduction?

Dear Sir:

We read with interest 2 articles published in the November 2005 issue of the Journal (1, 2). Both studies showed that a low-fat diet (≈30% total energy from fat) lowered LDL-cholesterol concentrations by ≈7–10% in the short term (6 wk and 3 mo, respectively). However, there were some remarkable differences between the studies: HDL cholesterol decreased by 7% and triacylglycerols increased by 14% in the 6-wk trial (1), whereas there was no change in HDL cholesterol and a 12% decrease in triacylglycerols in the 12-wk trial (2). One possible explanation for the contrasting effects on the concentrations of circulating HDL cholesterol and triacylglycerol of the 2 similar low-fat diets, apart from the obvious difference in diet duration (6 wk compared with 3 mo), may be the constant weight the participants maintained throughout the 6-wk trial. In contrast, there was a mean reduction in body mass index (in kg/m^2) of 1.2 (corresponding to ≈2 kg) in the subjects who followed the 3-mo diet. As pointed out by Ordovas (3), when low-fat diets produce weight loss, their detrimental effects (ie, reducing HDL cholesterol and increasing triacylglycerols) may no longer be evident.

Vincent-Baudry et al (2) showed no significant benefit of a Mediterranean-style diet on blood lipids compared with a standard low-fat diet, which led to the conclusion that both diets (Mediterranean-type and low fat) significantly reduced blood lipids and the calculated cardiovascular disease risk factors to an overall comparable extent. We think this message may underestimate the real advantage of the whole dietary approach in managing chronic disease. First, the follow-up may have been too short to detect a significant difference: for instance, in our study that compared a Mediterranean-style diet with a low-fat diet in subjects with the metabolic syndrome (4), the 2-y net change in lipid concentrations favored the Mediterranean-style diet [median (95% CI) changes: total cholesterol: −0.23 mmol/L (−0.44, −0.02 mmol/L); HDL cholesterol: 0.07 mmol/L (0.02, 0.14); and triacylglycerols: −0.21 mmol/L (−0.36, −0.06)]. Second, the positive effect of a Mediterranean-style diet on cardiovascular disease risk was also shown in the absence of any effect on blood lipids (5). Lastly, the global cardiovascular disease risk burden is composed of many components, of which lipids represent a huge part. However, the diet-lipids-heart hypothesis seems too reductive because it excludes many effects of diet on old and new risk factors, including blood pressure, endothelial function, vascular inflammation, insulin sensitivity, and oxidative stress (6).

The authors had no conflicts of interest.

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Reply to D Giugliano and K Esposito

Dear Sir:

Giugliano and Esposito are correct in noting that in our study (1) HDL-cholesterol concentrations declined, whereas triacylglycerol concentrations increased, when participants were fed diets that were low in total fat and saturated fat and that these

results differed from those observed by Vincent-Baudry et al (2), whose study compared a low-fat diet with a Mediterranean-type diet. The changes observed in our study are consistent with those observed in many other controlled feeding studies and agree with the changes predicted in a meta-analysis (3).

Giugliano and Esposito offered that the differences in weight loss between the studies may underlie the differences in the HDL-cholesterol and triacylglycerol responses. We do not disagree that weight change associated with low-fat diets may influence the resulting lipoprotein responses, as has been summarized by others (4). Indeed, we specifically chose for the participants to maintain a constant weight during the study so that we could examine the effects of genetic and nongenetic predictors of individual lipoprotein responses on diet without the confounding influence of varying degrees of weight loss.

It is worth noting, however, that other study design differences may have also played a role in defining the differences in the HDL-cholesterol and triacylglycerol responses. As mentioned, our study was shorter in length (6 wk compared with 3 mo). Additionally, our study examined only healthy men, whereas the study by Vincent-Baudry et al studied both men and women with at least one risk factor for cardiovascular disease. Importantly, our study was a controlled feeding trial in which all diets were chemically validated before the study and all foods were prepared and provided to the participants (with the exception of a weekend evening meal). Thus, in our study, there was a high degree of confidence that the diets consumed by our participants achieved the differences in total fat and saturated fat specified by the study design. This contrasts with the study by Vincent-Baudry et al, where diet change was achieved through counseling and validated by food records and 24-h dietary recalls. Differences associated with the interpretation and implementation of the dietary instructions, along with errors associated with diet reporting, provide less confidence that the designed differences in dietary fat intakes were, in fact, achieved or accurately reported in that study. The net effect is that the reported change in dietary fat intake in the persons who were counseled to consume a low-fat diet was approximately half that achieved in our controlled dietary study (−4.9% compared with −8.7% of calories on the Step I diet). Given the dependence of the changes in HDL cholesterol and triacylglycerols on changes in total fat intake, it is likely that the differences in achieved reductions in fat intake between our studies also contributed to the differences in outcomes.

The study conducted by Vincent-Baudry et al is immensely important in that they tested whether the benefits (or adverse effects) of a particular dietary approach, which are identified through controlled feeding studies such as ours, were realized under real-world conditions. However, because the goals and resulting experimental designs of the studies are often quite different, it can be difficult to precisely identify those factors that may be responsible for any apparent discrepancies in observed outcomes. This is best resolved through additional carefully designed and executed studies of both types.

The authors had no conflicts of interest.

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