British Journal of Nutrition (2011), **106**, 959–960

Letter to the Editor

Response to Clifton

(First published online 17 June 2011)

We thank Dr Clifton for his interest in our paper[1]. We would like to again clarify the central finding of our analysis, that mixed *n*-3/*n*-6 PUFA interventions had significantly different CHD effects compared with interventions selectively increasing *n*-6 linoleic acid (LA) ($P=0{\cdot}02$), determined using standard methodology for performing and interpreting heterogeneity analysis[2]. As discussed in our paper and in our response to Harris *et al.*, the mixed *n*-3/*n*-6 PUFA intervention category contains two trials that increased EPA + DHA alongside LA (EPA + DHA + LA interventions), and two trials that increased α-linolenic acid (ALA) and LA without concurrent increases in EPA + DHA (ALA + LA interventions). Because no significant heterogeneity exists between the two categories of mixed *n*-3/*n*-6 PUFA interventions ($Q=0{\cdot}22$, $df=1$; $P=0{\cdot}64$), we did not previously report a comparison of LA-selective PUFA interventions with ALA + LA interventions in heterogeneity analysis. However, such an analysis performed in response to this letter demonstrates that LA-selective and ALA + LA interventions have significantly different effects on CHD risk with ($Q=5{\cdot}96$, $df=1$; $P=0{\cdot}01$) or without ($Q=3{\cdot}95$, $df=1$; $P=0{\cdot}047$) the inclusion of the Sydney Diet Heart Study (SDHS). This finding again emphasises the necessity of making a clear distinction among PUFA species, and further demonstrates that CHD benefits of interventions increasing both *n*-3 and *n*-6 PUFA are not necessarily attributable to *n*-6 LA.

Although we did not make the following conclusion in our paper, Dr Clifton attributes the conclusion that 'interventions which contain ALA as well as LA show benefit whereas those which contain LA alone do not show benefit' to us. We would therefore like to further clarify our findings here. Pooled analysis of all four *n*-6 LA-specific datasets (*n* 9569) shows a relatively consistent, albeit non-significant, signal towards increased risk for CHD death (+28 %, risk ratio (RR) 1·28, 95 % CI 0·96, 1·71; $P=0{\cdot}09$), total CHD events (+23 %, RR 1·23, 95 % CI 0·94, 1·61; $P=0{\cdot}13$) and death from all causes (+16 %, RR 1·16, 95 % CI 0·95, 1·42; $P=0{\cdot}15$). Although these findings point towards possible harm rather than benefit, the pooled 95 % CI include 1·0, therefore one should not definitively conclude that *n*-6 LA-specific PUFA interventions provide no benefit or cause harm. This statistical uncertainty was appropriately reflected in our conclusion stating that interventions increasing *n*-6 LA in place of *trans*-fatty acids (TFA) and SFA provide 'no indication of benefit, and there is a possibility of harm' and our abstract stating that 'advice to specifically increase *n*-6 PUFA intake [is] unlikely to provide the intended benefits, and may actually increase the risks of CHD and death'. By contrast, pooled analysis of the two mixed *n*-3/*n*-6 PUFA interventions that increased ALA + LA without also increasing EPA + DHA shows a non-significant trend towards CHD benefit (RR 0·81, 95 % CI 0·64, 1·02; $P=0{\cdot}07$), rather than the signal towards harm of LA-selective PUFA interventions. Importantly, however, ALA was accompanied by large amounts of *n*-6 LA in both trials, as well as in a recent highly publicised negative trial[3] that used a high-LA margarine (39·8 g LA/100 g) to deliver ALA (13·7 g ALA/100 g). Because the potential metabolic benefits of ALA could be masked by the presence of large amounts of *n*-6 LA[4,5], these data should not be interpreted as ruling out the possibility of CHD benefits specific to *n*-3 ALA. We have no randomised controlled trial (RCT) data to evaluate the CHD effects of increasing ALA without concurrent addition of much larger amounts of LA. This critical research gap could be resolved by an RCT substituting conservative amounts of flaxseed oil (not soybean oil or ALA + LA margarine) for other fats with the evaluation of CHD outcomes.

The Minnesota Coronary Survey (MCS)[6] provided the largest number of CHD events (252) and deaths (517) of any RCT, despite the low event rate specified by Dr Clifton. Importantly, intent-to-treat analysis of the full dataset showed more CHD events in the LA-specific PUFA group, especially in women (RR 1·31, 95 % CI 0·90, 1·90); these numbers include a curious twofold increase in CHD events (RR 2·15, 95 % CI 1·19, 3·87; $P=0{\cdot}01$) in women on the LA-specific PUFA intervention for 1 year or less. Although the LA-specific PUFA group remaining in the study for >1 year had slightly fewer CHD events, this subgroup excludes the LA-specific participants who had already experienced a CHD event within the first year (perhaps the most vulnerable group). MCS investigators appreciated in 1968 that the duration necessary to observe CHD effects 'could be a long time, or the effect might very well appear immediately, if all that is required is arrest of the process, or if the mechanism of the effect is in large part through the clotting phenomenon'[7]. Since dietary fatty acids alter thrombosis, arrhythmogenesis and inflammatory processes, in addition to lipoprotein metabolism and oxidation, and an alternative analytical plan was not specified[8], it is most appropriate to use the 'gold standard' intent-to-treat methodology in evaluating CHD outcomes in all randomised participants.

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We agree that TFA probably played a key confounding role in these RCT, a point overlooked in previous meta-analyses but discussed at length in our paper[1]. It is important to note, however, that the liquid vegetable oils and cholesterol-lowering (soft) polyunsaturated margarines consumed by the experimental dieters generally contain more LA, and considerably less TFA[9], than the shortenings and common (hard) margarines consumed in moderate-to-large quantities by the control groups. RCT with experimental margarines observed that they were specially formulated to be 'soft' or 'polyunsaturated'[1,8,10,11], or that the 'major component is unaltered vegetable oil with a small amount of hydrogenated fat added as a hardening agent'[12]. Likewise, the 'Miracle' brand margarine used in the SDHS was a polyunsaturated product formulated in 'consultation with leading hospital authorities, physicians and dietitians'[13]. Hence, because the experimental diets probably contained substantially less TFA than control diets, the reported CHD benefits of mixed *n*-3/*n*-6 PUFA interventions may have been overestimated, and the potential harm of *n*-6 LA-specific PUFA interventions may have been underestimated, as noted in our paper[1].

We agree with Dr Clifton that important evidence gaps remain, and have outlined the design of an RCT to evaluate the specific effects of lowering *n*-6 LA on clinical CHD outcomes in our paper and in our response to Harris *et al.* The results of such a trial will have major public health implications and should put this debate to rest once and for all.

Acknowledgements

The study was supported by the intramural research programme of the National Institute on Alcohol Abuse and Alcoholism. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute on Alcohol Abuse and Alcoholism or National Institutes of Health. The authors declare that they have no conflicts of interest.

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doi:10.1017/S0007114511001668

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