Author's response to reviews

Title: No evidence of differential effects of SFA, MUFA or PUFA on post-ingestive satiety and energy intake: a randomised trial of fatty acid saturation

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Version: 2 Date: 7 March 2010

Author's response to reviews: see over
Dear Nutrition Journal Editorial Team,

Re: No evidence of differential effects of SFA, MUFA or PUFA on post-ingestive satiety and energy intake: a randomised trial of fatty acid saturation.

We thank the reviewers for their comments on this manuscript. Please find below a point by point response to each of the comments and requests made by the four reviewers. The changes have been highlighted within the manuscript to aid further review.

In addition, the trial registration number [ACTRN12610000193077] has been cited in the manuscript on Page 3, as requested.

We appreciate your consideration of this revised manuscript for publication by Nutrition Journal

Kind regards

Associate Professor SD Poppitt
Minor Essential Revisions

1. In order to further clarify Figure 1, which shows indicators of palatability of the breakfast meals for each of the 3 treatments, the methods section has been edited on page 8 lines 21-23. In addition the results on page 11 lines 19-23 and the Figure Legend on page 26 has been edited for clarity.

2. In Table 4, page 30, which presents data from previous studies investigating effects of fatty acid saturation on measures of appetite, we have edited ‘study endpoint’ column and also included an additional column ‘study outcome’ to make clear the outcomes in each study reviewed.

Discretionary Revisions

1. We thank the reviewer for the comments on energy density and agree that this is a critical factor that influences spontaneous energy intake (references 10, 11, 15-17 in the Introduction, page 4, line 9). We also note the reference by the Blundell research team on passive overconsumption (reference 10):

Reviewer #2_Moorhead

Minor Essential Revisions

1. As noted by the reviewer only 17 of the registered 18 participants completed the study. One individual left New Zealand during the study, withdrawing after 2 of the 3 treatment visits. The treatments completed were SFA and MUFA; the PUFA treatment was not completed. This information has been included on page 11, lines 3-6. The trial was analysed as an intention to treat (ITT) hence all data from all treatment visits completed were included in the analysis (ie. data from the SFA and MUFA treatments from the withdrawn patient were included in the analyses). This has also been clarified on in the statistical Analyses section on Page 10, lines 2-5.

2. The Figure legends have been revised to include the participant numbers. See Figure 1, Figure 2, Figure 3 (page 26)

3. The text has been revised and ‘participant’ is now used throughout. Changes have been highlighted in the text.

4. We have reviewed Table 4 and re-edited to ensure that all of the information is clearly visible and doesn't cut across any of the lines of the Table.

5. We have revised Figure 1 – thank you for the note re error bar cut-off.

6. Figure 3 has also been revised re missing bracket.

Discretionary Revisions

1. The Introduction (lines 2-6) has been revised in order to shorten and clarify some of the sentences as suggested by the reviewer: “A high intake of dietary fat has long been implicated in the development of obesity with a positive association between a high-fat (HF), high-energy dense diet and a high body mass index (BMI) (Lissner and Heitmann 1995; Bray, Paeratakul et al. 2004). Whether there is a causal relationship between dietary fat and the current high levels of obesity continues to be debated however, (Willett 1998; Astrup, Grunwald et al. 2000; Hill and Astrup 2003; Pirozzo, Summerbell et al. 2003; Astrup 2006;
Field, Willett et al. 2007) as does dietary fat as a primary driver of hyperphagia, or overconsumption, during weight gain.

2. ‘Years’ has been added on page 6, line 3

3. We have included typical food portions (No. of typical serves, column 2) in Table 2

Reviewer #3_ Levitsky

The reviewer has raised a number of important issues arising from the submitted manuscript and we appreciate the insight of these comments. We have considered all of these points and have revised the manuscript to provide further explanation and with the intent to improve the content and structure of the text. Changes and insertions made in the manuscript have been highlighted throughout for aid of further review.

To respond to each query and issue in turn:

1. It is correct that the primary aim of the trial was to determine whether postprandial measures of appetite regulation were significantly different between lipids with varying saturation profiles. There are possibly a number of designs that could address this question including of course the 4 treatment design (with a control ‘no fat’ or a ‘no energy’ treatment) outlined in the reviewer’s report. The addition of a ‘no fat’ or ‘no energy’ control treatment ensures that the method used is sufficiently sensitive to detect physiologically important effects within a trial. In a previous trial we investigated the sensitivity of our current method to manipulations of energy and fat content, in a 4 treatment paradigm [3 x active treatment plus ‘no energy’ control (Lithander, Strik et al. 2008)], using a protocol and subject group identical to the trial that we are currently reporting. The earlier trial showed that this acute postprandial appetite assessment method is sensitive to comparisons between high fat/high energy breakfast treatments and a ‘no energy’ control.

   Hence, in the current study we used a 3 treatment cross-over to ask the questions:

   - are subjective VAS-assessed measures of satiety and/or measures of spontaneous energy intake at a subsequent meal significantly different between high-SFA, high-MUFA or high-PUFA meals which are balanced for fat content, energy content and energy density?

   and if so,
can post hoc interrogation determine which, if any, of the 3 saturation profiles generates (i) greater or less hunger, and/or (ii) higher or lower intake at the ad libitum outcome meal?

This 3 treatment design has been previously used in similar studies of short-term appetite regulation by a number of research groups including (Lawton, Delargy et al. 2000; Kamphuis, Westerterp-Plantenga et al. 2001; Flint, Helt et al. 2003). The current study was based on the published design of Flint, Astrup and colleagues (Flint, Helt et al. 2003) in a trial designed to compare the effects of high PUFA, MUFA and trans fats on acute appetite response, and lipid emulsion studies of Burns and colleagues (Burns, Livingstone et al. 2000; Burns, Livingstone et al. 2001; Burns, Livingstone et al. 2002).

Whilst there was no significant between-treatment effect for the measured outcomes, it is clear from the VAS data that, as expected, there was a strong within-treatment response to each of the 2MJ high-fat breakfasts (ANOVA, time, P<0.05, 0-210 mins). Hunger & prospective consumption significantly decreased from the fasting baseline whilst fullness & satisfaction significantly increased immediately following consumption of each of the high-SFA, MUFA and PUFA treatments; all satiety measures then gradually returned to baseline. Whilst there was no evidence of a differential effect between treatments it is clear that the experimental paradigm induced large and reproducible effects during the post breakfast period, and we have included information on the significant within-treatment changes on page 12, lines 3-5.

We do however agree that the active treatment + control is a design used consistently by the pharmaceutical industry in placebo controlled trials, and was also used in several trials previously investigating the effect of f.a. saturation on appetite (French, Conlon et al. 2000; Alfenas and Mattes 2003; MacIntosh, Holt et al. 2003; Burton-Freeman 2005; Feltrin, Little et al. 2008). Whilst this inclusion does extend interpretation of the trial outcomes in a situation where no differential main effects are observed, we do not think that the absence of this treatment arm renders the trial uninterpretable. The choice of an appropriate ‘placebo’ treatment in macronutrient manipulation studies can be difficult. Selecting a placebo which is either a ‘no energy’ or ‘no fat’ (and hence high CHO and/or protein) control introduces factors other than fatty acid saturation into the experiment and may drive observations of significant between treatment effects when analysed by ANOVA. Whilst these effects are of course real, we were not primarily investigating in this trial whether high-fat SFA, MUFA, or PUFA meals have a greater effect than a ‘no energy’ or ‘no fat’ meal.

The comparison of fat types in this trial is modelled on pharmaceutical methods of bioequivalence comparison studies where it is common practice to compare directly between treatments rather than versus a placebo control.

In the Conclusions section (page 17, lines 13-15) we have been cautious in the wording of the trial findings, in light of the experimental design. “In this study we were unable to show differential changes in postprandial feelings of hunger or fullness, or changes in energy intake at a lunch meal when alterations were made to the fatty acid saturation of a high-fat breakfast”.

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2. It is also correct that the participants in this trial were cannulated prior to baseline, and sequential blood samples collected throughout the experiment. This was carried out for 2 reasons. Firstly, with the intent of investigating putative appetite-related mechanisms such as postprandial peptide response (e.g., CCK, GLP-1) should a differential effect of SFA, MUFA or PUFA be found during the observational study. Secondly, the cannulation and blood procedures were used as a distraction for the participants since this was a covert manipulation (see Page 7, lines 21-22). Participants were not informed until completion of the trial that the primary focus of the study was measurement of food intake at the lunch meal, rather they were told that putative changes in serum triglyceride in response to different fat profiles was the primary outcome.

Analysis of the biochemistry (lipids, glucose, insulin) showed no significant difference between treatments and in view of generating a concise text in this ‘no evidence of significant effect’ trial we did not include this data. In the revised manuscript we have now inserted information re these non-significant effects on Page 12, lines 14-17: “Analysis of the sequential blood collects also showed there to be no difference between circulating levels of serum cholesterol, triacylglycerol (TAG), glucose or insulin between the SFA, MUFA and PUFA treatments (all, treatment*time P>0.05)”. Figures presenting the blood plots can certainly also be included in the revised manuscript if the reviewer considers this warranted.

3. The introduction has been revised on Page 5 lines 10-16, to clarify the hypotheses in this trial: “This lack of consensus led to our current study which investigated whether changes in the saturation profile of a high-fat breakfast meal affected postprandial appetite sensations and food consumption at the subsequent meal using a study design which we have previously shown to be sensitive to manipulations in energy and fat content (Lithander, Strik et al. 2008). Based on prior studies we hypothesized that there may be a satiety hierarchy of PUFA>SFA>MUFA when a high fat meal balanced for both energy and total fat content is consumed”

4. In response to the reviewers’ comments the Discussion on pages 14-17 has been significantly redrafted to clarify and improve the reader’s understanding. In particular sections of pages 16, 17 have been redrafted re discussion of prior trials: "Some variability in outcome may in part be due to differences in methods. Important aspects of trials assessing postprandial changes in appetite may include number and characteristics of participants; lipid dose, composition and route of administration; inter-meal interval; and composition and variety of foods offered at the ad lib outcome meal. These have been summarized in Table 4. Of the trials so far conducted, all were small sample studies (8-25 participants) conducted predominantly in lean, healthy participants. Dose of lipid administered does not appear to unduly bias outcome since studies by Lawton (Lawton, Delargy et al. 2000) and Flint (Flint, Helt et al. 2003) both administered high doses of lipid (>50g fat), yet only the Lawton study conducted in lean participants showed differential effects of f.a. saturation. The fact that delivery of lower doses, such as our current trial (26g), are sufficient to elicit a response was demonstrated by Kamphuis and colleagues where a 25g lipid supplement differentially altered EI (Kamphuis, Westerterp-Plantenga et al. 2001). Burton-Freeman (Burton-Freeman 2005) administered a low dose (9-13g) lipid treatment yet observed significant effects on some VAS-assessed satiety ratings, albeit these did not result in suppression of EI at a subsequent test meal. The route of lipid administration is likely to also be important and it is notable that 2 (French
2004; Feltrin, Little et al. 2008) of the 4 (Lawton, Delargy et al. 2000; Kamphuis, Westerterp-Plantenga et al. 2001) trials where a differential response was elicited delivered the lipid treatments via GI infusion hence bypassing early sensory and cognitive signaling. Whilst it is difficult to find methodological issues directly responsible for the variable outcome of the studies to date, each of the trials appears robust in its approach and it is clear that a strong case cannot be built for enhanced satiety with high-SFA, MUFA or PUFA based on current evidence.”
1. Abstract, lines 15-16. We have included information that VAS were also completed before the test breakfast.

2. Abstract, line 22. This has been revised to prospective food consumption.

3. The Introduction has been revised to shorten and clarify the sentences in the first paragraph: “A high intake of dietary fat has long been implicated in the development of obesity with a positive association between a high-fat (HF), high-energy dense diet and a high body mass index (BMI) (Lissner and Heitmann 1995; Bray, Paeratakul et al. 2004). Whether there is a causal relationship between dietary fat and the current high levels of obesity continues to be debated however, (Willett 1998; Astrup, Grunwald et al. 2000; Hill and Astrup 2003; Pirozzo, Summerbell et al. 2003; Astrup 2006; Field, Willett et al. 2007) as does dietary fat as a primary driver of hyperphagia, or overconsumption, during weight gain”

4. Page 4 lines 21-23 and page 5: this paragraph has been reworded to clarify the information on previous food intake studies. “Whilst there is some evidence of PUFA having the strongest and MUFA the weakest suppression of food intake, clinical studies are inconsistent and variable. A gastrointestinal infusion study showed high-PUFA Intralipid® (a fat emulsion comprising largely soybean oil) and linoleic acid (18:2) to decrease intake when compared with a no-fat saline control, whilst carbon chain length (CCL) -matched stearic acid (C18:0, SFA) and oleic acid (C18:1, MUFA) did not (French, Conlon et al. 2000). In a feeding study of CCL-matched fats, both high-PUFA and high-SFA meals decreased food intake when compared with high-MUFA meals (Lawton, Delargy et al. 2000), and a second infusion study showed lauric acid (C12:0 SFA) but not the longer chain oleic acid (C18:1 MUFA) to decrease food intake relative to a saline control (Feltrin, Little et al. 2008)”

5. Page 5, line 9: ad libitum has been revised.

6. The intent in this study of lean male participants was to exclude individuals who showed a significant history of food and/or body weight-associated events. This included dieting for weight loss, eating disorders and also individuals who showed evidence of marked restraint. At the screen visit several questions were asked around current and/or history of dieting (dieting within the previous 6 months was an exclusion), and the Stunkard and Messick 3 factor eating behaviour questionnaire was given to the participants. Those who scored 12 or greater on the restrain aspect of Stunkard questionnaire were not randomised into the study. The issue of restraint is of course of great interest in studies of appetite regulation, particularly those acute
studies carried out within a research facility, such as our current trial and outcomes are mixed (eg. Martins et al Proc Nut Soc 2008). Indeed, interpretation of restraint scales and the effect of using this classification for appetite studies does still remain under debate (eg. Stice et al Psychol Assess. 2004; Stice et al Appetite Dec 16 2009, e-pub). Hence in line with many previously published trials, we chose to conduct our trial in male subjects who reported low restraint on the Stunkard scale. We have revised the methods section of the text (page 6, lines 6-9) and added this reference to better explain the process that we used and the reasons for exclusion.


7. The comment re energy density on page 14, line 20-22 has been revised to make it clear that this does refer to data from our current study where as noted by the reviewer there were small differences in energy density of the test muffins. ED has now been included in Table 1. It is notable that these were high-fat (50 en%), high ED (13.1, 12.9, 13.4 kJ/g) breakfasts. Please also note that in Table 1, that weight of treatment has been revised from raw ingredient weights to cooked weight, to allow reporting of ED of each test item as consumed. Weight of muffins was SFA=151g, PUFA=151g, MUFA=145g

8. Page 7, line 11. The 200mL water was given to the subjects when they arrived at the Unit in order to standardise amounts consumed prior to the breakfast. Previous studies that we have run have shown that the participants varied considerably in their water consumption prior to attending the early morning clinic. As data is mixed as to possible effects of water-induced gastric distension, the 200mL standard drink was included in the protocol.

9. A cannula was inserted in all participants with the intent of investigating putative appetite mechanisms such as postprandial peptide response (eg. CCK, GLP-1) should a differential effect of SFA, MUFA or PUFA be found in the observational part of the study. The lack of effect prevented these analyses being performed. Biochemistry, including lipids, glucose and insulin, was analysed but no significant differences were found. Reference to these non-significant effects has been inserted on Page 12, lines 14-17.

10. The reviewer is correct that the data presented on physical activity on the day prior to the clinic visit (Day -1) was from the PEPSA scoring system. Reference to LINZ has been removed on Page 7.
11. The mismatch in analytical methods has also been revised. All data has now been analysed using SAS, including the Day -1 reported energy intake and physical activity. This is specified on Page 10, lines 2-4.

12. The information given on Page 10, lines 4-5 is in reference to the intention to treat (ITT) analysis that was performed. All of the data collected in this trial was analysed, including the subject who withdrew from the trial after 2 treatments. There are several statistical methods that could be used to deal with this withdrawal and subsequent missing data point(s), (including of course analysing only the data from subjects who completed = completers only). One way to statistically address the issue of subject drop out is to analyse the data as an ITT (ie, all available data), assume that the data is missing at random and to not impute any missing data. Other valid statistical options would be to impute the missing data using one of the several replacement methods published, such as (i) insert the mean value for the n=17 subjects for whom there is data or (ii) carry the last value of the drop out subject forward (=LVCF, last value carried forward). To evaluate this data analysis and the effect of the drop out subjects we also conducted a ‘completers only’ analysis with n=17 subjects for each of the 3 treatment arms, and confirmed that the method of statistical analysis had no effect on the outcome of the study, ie. no difference between treatments for any of the variables when analysed as completers only.

13. Page 15, we have revised the Discussion to clarify between study effects. See Pages 15, 16, 17.

14. Conclusions, page 17, line 17, we have edited this sentence to replace bolus with ‘test meal’ as suggested “In this study we administered a 26g lipid test meal”

15. We have revised Table 4 in line with the reviewers’ suggestions to include further detailed information on the methods for all of the published trials. We note that some method details had been omitted and these have now been included in the Table. We have also edited the ‘Study Endpoint’ column for clarification, and inserted an additional column on the far right of the Table called ‘Study Outcome’. This details the changes in ad lib energy intake that were reported for each of the individual studies to allow the reader to easily review effects of lipid treatments across the studies.
References