Author's response to reviews

Title: Recent findings on the health effects of omega-3 fatty acids and statins, and their interactions. Do statins inhibit omega-3?

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Author's response to reviews: see over
ANSWERS TO THE REVIEWERS

REVIEWER 1

1- “The authors have provided an excellent analysis of recent randomized controlled trials of omega-3 polyunsaturated fatty acids (n-3 PUFAs). As the authors note that majority of more recent trial have not confirmed the cardiovascular benefits of n-3 PUFAs reported in the previous trials. They then center on the possible effects of the increased use of statins for the management of cardiovascular disease for apparent diminished efficacy of n-3 PUFAs in the more recent trials. Specifically, they propose that the negative/neutral finding resulted from negative interaction between the statins and n-3 PUFAs - statins counteract the actions n-3 PUFAs - and in themselves increase rather than decrease the risk of adverse cardiovascular events. The authors also suggest that n-3 PUFAs may only have benefits in n-3 PUFA deficient patients. They conclude by presenting a few mechanisms by which statins could adversely affect cardiovascular health. The authors’ hypothesis is interesting and clinically important. This hypothesis certainly merits further investigation. However, an important limitation of the present review is the authors somewhat selective sampling of the literature.”

Thank you very much for these comments.

Regarding our “selective sampling of the literature”, we would like to underline that the present article is not a “systematic review”. It is a summary of recent findings, as indicated in the title, focusing on the clinical effects of n-3. We are however ready to add some useful references (see below).

2- “Major compulsory revisions: 1. The authors also need to discuss recent clinical and animal studies that not only failed to confirm a beneficial effect of n-3 PUFA but report that this treatment increased rather than decreased adverse cardiovascular events. For example, Burr et al. (Eur J Clin Nutr 57:193-200, 2003) reported that n-3 PUFAs increased, rather than decreased, all-cause mortality (15% increase over 9 year follow-up period, with a 54% increase in sudden death).

The study by Burr et al – the DART-2 trial – published in 2003 is not really “recent”. More importantly, there were many design issues in that trial – for instance, the one year interruption followed by a nonconventional re-randomization – with different numbers of patients in each of the 4 groups (see table 1 in the article) – a lack of true control group, a lack of placebo to compare with the fish oil capsules, and a total lack of “blinding”.

According to the authors, it is the use of capsules of fish oil rather the intake of n-3 under the form of fish (see Table 8 in the article of Burr) which might have been responsible of the “apparent” increase in cardiac death and not the n-3 themselves. The authors write in their discussion: “The apparently adverse effect of fish advice was confined to the second phase of the trial (data not shown), when a much higher proportion of subjects were given fish oil capsules than in the first phase”.

This may question the quality (safety) of the fish oil used in these specific capsules.

As our decision was to only retain high quality trials respecting the fundamental rules of scientific medicine in our analysis, we felt inclined not to include results of a trial with so many “technical” problems. We think that the results of the trial are not interpretable and therefore that the best solution is to exclude DART-2 from the present analysis.

However, in the revised version of the manuscript, DART-2 is now cited (ref 30) but not discussed (beyond the design issues).
3- In a similar manner, Raitt and co-workers (JAMA 293:2884-2891, 2005) reported that fish oil supplements not only did not reduce ICD events or mortality but also increased arrhythmic events in the subgroup of patients (67%) who received an ICD with ventricular tachycardia as an indication. [The authors have presented this study but have not addressed this possible proarrhythmic effects reported in sub-group of the patients, that admittedly was not confirmed in subsequent studies]”

Actually, Raitt et al reported no effect of 1.3 g/day of EPA+DHA in patients with a ICD. In a subset of 133 patients, there was an apparent increase in the risk of arrhythmias in the n-3 group compared with the placebo group. The problem with that trial is not only the lack of confirmation of the potential adverse effect of n-3 in subsequent trials, as commented by the referee, but mainly the very small sample size of the whole study (n=100 per group) in the context of major clinical heterogeneity – about one third of the patients had non ischemic heart disease. This makes secondary analyses questionable.

Thus, we concluded that the possibility of adverse effects of n-3 is not reliably evidence-based in the Raitt’s trial.

4- “Heart failure patients (NYHA class II and III) with the highest RBC n-3 PUFA levels also exhibited an increased risk for ventricular arrhythmias that required anti-tachycardic therapy (Am Heart J 155:971-977, 2008).”

This is an interesting study, but very different from the randomized trials we have included in the present analysis: it is a very short (one year) and very small observational study (n=102); with again a major clinical heterogeneity since about one third of the patients had non ischemic heart disease. For similar reasons as for DART-2, the consensus was to exclude this study from the present analysis.

Thus, in our opinion, none of the three studies cited by the referee provides evidence that n-3 are proarrhythmic in humans

5- “Finally, and perhaps most importantly with regards to the authors hypothesis concerning the negative interactions, are some recent animals studies. Coronel et al. (Cardiovasc Res 73:386-394, 2007) found that dietary n-3 PUFA increased the incidence of VF during regional ischemia in isolated pig heart preparations. More recently, Billman et al. (Circulation Arrhythm Electrophysiol 5:553-560, 2012) using a well-characterized canine model of sudden cardiac found that n-3 PUFA treatment (1-4 d/g/day for 3 months) produced large increases in the omega-3 index of both red blood cell and cardiac tissue yet not only failed to prevent ischemically-induced ventricular fibrillation but actually significantly increased the susceptibility to malignant arrhythmias in low risk dogs (both dogs with and without myocardial infarction-MI). Long-term dietary n-3 PUFA treatment induced ventricular tachyarrhythmias in one third of the post MI dogs that had been previously been shown to be resistant arrhythmias while two non-infarcted dogs died spontaneously during the 3-month n-3 PUFA treatment. This latter observation is particularly noteworthy, as these non-infarcted dogs would normally exhibit a negligible risk for sudden death. Statins were not used in either the porcine or canine study. Thus, statins mediated inferences with the action of n-3 PUFA cannot explain the possible pro-arrrhythmic actions of n-3 PUFAs in these studies.

These are very interesting comments.

Evidently statins do not play any role in these animal observations.

Our analysis does not focus on experimental cardiology although we agree that experimental studies often help understanding human data. Regarding n-3, however, we are afraid that the addition of confusing animal data to confusing human data would not help. As you write in page 557 of your excellent article (cited above) “variable results have also been reported in
animal studies”. This is true in studies on arrhythmias as well as in those on ischemia-reperfusion injury.

In a similar way as for the clinical studies discussed above, variable results in animal experiments can be often explained by the poor quality of many studies. Among the high-quality studies, in addition to yours, you mention the studies by Ruben Coronel in Amsterdam. As you know, this author published – the same year 2007 – data on the effects of n-3 in isolated pig hearts (promotion of arrhythmias, Cardiovascular Res 73:386-394) and in pig ventricular myocytes (reduction of the incidence of arrhythmias, Heart Rhythm 4:1452-1460). What could clinicians do with that?

Coming back to your recent work, your observation that dietary supplementation with n-3 – in contrast with your prior work examining the effects of n-3 infusion (ref 7 of the present manuscript) – significantly increases the susceptibility to malignant arrhythmias in a model of ischemia-mediated arrhythmias raises the question of the mode of administration of n-3: intravenous vs. dietary administration. We greatly appreciate the section “Strengths and limitations of the study” in your article, in particular your comments about the experimental model and the human-equivalent doses of n-3. However, we do not totally agree with your interpretation of the data, in particular when you assume that the “spontaneous deaths” among low-risk dogs receiving n-3 resulted from arrhythmias provoked by n-3.

Alternative explanations are not unlikely. For instance, your data may also suggest cardiac and/or non cardiac toxic effects of the ethyl esters with various causes of death. Toxic effects of ethyl esters in animal studies have been reported and in our animal laboratory we stopped using ethyl esters for toxic reasons. Compared with ethyl esters, the same doses of n-3 given under the form of triglycerides were not toxic in rats. It is noteworthy that the proarrhythmic effect in your study was seen for the largest doses which reinforce the hypothesis of the toxicity of ethyl esters.

Finally, if a proarrhythmic effect of n-3 is not demonstrated – both in animal and human studies – it remains that some recent studies of dietary supplementation, including yours, did not confirm the previous antiarrhythmic effects reported when using n-3 infusion.

We cannot decide whether it is due to a lack of effect or to a “light” adverse effect. A short sentence has been added (and your article is now cited, ref 32) in the revised manuscript.

6- “Minor Essential Revisions: Page 2, line 12: either the results were significant or there were not, eliminate the phrase about “borderline non-significant protection”

Terminology is indeed important. We agree that statistical comparisons based on an a priori hypothesis tested in a specific experiment are significant or not; and the word “borderline” is not appropriate in this case. However, when “interpreting” the results of meta-analysis – based on the pooling of data of several non-significant studies with the aim at raising new hypotheses or potential explanations – it is important to separate totally negative data from “borderline” significant or non-significant data.

In caricaturing a little bit, a risk ratio of 0.70 with 95% confidence intervals 0.500-1.001 (means “borderline” not significant) is not the same thing as a risk ratio of 1.002 with 95% confidence intervals 0.950-1.050. In the second case, it is better to reject (and forget) the tested hypothesis whereas in the first case, the probability that the trials included in the meta-analysis and the meta-analysis itself are underpowered is high and it might be justified to
launch a new trial with larger sample size and/or longer follow-up. In that case, the use of “borderline not significant” is appropriate in our opinion.

7- “Page 2, lines 13-15: should a read as “Finally, no significant protection was reported in two large RCTs (JELIS and GISSI) that combined a statin with n-3 treatment – suggesting a possible reciprocal inhibition.” To what GISSI are you referring, GISSI-HF?”

Thank you. The sentence is modified in the revised manuscript. Also we have added “HF” to GISSI to get GISSI-HF.

8- “Page 3, line 1: should read as “Until 2005, studies consistently provided clear evidence....”

OK. Thank you.

9- “Page 4, line 3-4: either the results were significant or there were not, eliminate the phrase about “borderline non-significant protection”

See our answer number 6. Here, it is about “secondary analyses”, but same reasoning as for “interpretation” of meta-analysis.

10- “Page 4, 4 lines from bottom of the page: “...and reported a non-significant effect...”

OK. Thank you.

11- “Page 4, last line: should read as “either groups” not “neither group”.

OK. Thank you.

12- “Page 5, line 10: “an ICD” not “a ICD”

OK. Thank you.

13- “Page 5, lines 16-17: should read as “... to detect protection...”

OK. Thank you.

14- “Page 8, line 18: should read as “...trial with single group receiving p only the placebo.”

OK. Thank you. But we must write “placebo” with an “s” because there were two different placebos

15- “Page 9, line 4: should read as “...was not protective.” Delete “at all”.

OK.

16- “Page 9, line 12: should read as “...in any biological mechanisms...”

OK

17- “Page 10, line 3: How was “lower cognitive function” assessed in a dog? Should merely state what test was impaired rather than speculate on a dog’s ability to think.”

OK. We now refer to “deficits in a task that measures executive function in statin-treated dogs”. We have added one reference (now ref 61) to support the concept.

18- “Page 11, line 3: should read as “Finally, this negative action on the central nervous system probably explains...”

OK. Thank you.
OK. Thank you.

20- “Page 12, first paragraph: A similar argument can be made concerning the n-3 PUFA studies. A potential conflict of interest is also possible for n-3 PUFAs, as there is a huge and growing market for n-3 PUFA supplements.”

We agree with that and we have added a few words at the last sentence of page 14 (revised version): “Only scientists and physicians free of conflicts of interest and independent from the industry – both the n-3 supplement and statin industries – should be invited at ...”

OK. The sentence has been slightly modified in the revised manuscript.

We now write: “... the almost total innocuousness of n-3 in most populations ...”

On the basis of human and animal data, we actually think that there is no firm evidence of proarrhythmic effects of n-3. However, we are afraid that some marketed fish oil may not be totally safe, in particular the one used in DART-2. Also, we think that high doses of ethyl esters should be contraindicated ... It remains to decide which doses are “high” (?)

We thank the referee very much for his help in improving the manuscript.

REVIEWER 2

1- “de Lorgeril et al provide an article that is partly review, partly hypothesis. The authors propose an interaction of statins with n-3 fatty acids, and discuss the pros, but hardly the cons, of this concept. The manuscript is very strongly opinionated, and, in parts, does not conform to scientific standards.”

These are interesting comments. Our article is actually a review – of recent and sometimes quite confusing data about n-3 – aiming to propose a unifying theory to explain them and help physicians to make adequate decisions in everyday medicine.

This is the job of the scientist: interpreting study results and proposing new theories to be discussed by the scientific community and eventually tested in future studies.

Contrary to your statement, we believe that our article is only slightly “opinionated”.

“Interpretation” is not “opinion” when it is based on reported facts! It is important to make the difference. Finally, we will show in the next paragraphs that the present text actually “ conforms to scientific standards”.

2- “Major comments. The direct comparison of fatty acid measurements from different labs is not possible. What is 1% in one lab, may be 1,5% in another. The lab-to-lab variability was found to be that large (Harris WS et al, unpublished).”

After 30 years of research activity about fatty acids and their measurements in various conditions, we are aware that there is some lab-to-lab variability; and we take that issue into account when we interpret data from different studies. Your remark actually signifies that we should never compare studies with other studies.
3- “Both OMEGA and SU.FOL.OM3 were underpowered. By definition this precludes conclusions on the therapeutic effect of omega-3 fatty acids. It is unclear, whether the trial or the therapeutic effect was too small.”

Actually OMEGA and Sufolom3 were underpowered. Should the two trials therefore be rejected?

The main – maybe the only one – interest of a meta-analytic process is to pool data from underpowered studies to get sufficient statistical power and get information.

It was the purpose of the meta-analysis (of secondary prevention trials) published in April 2012 (ref 24 in the present manuscript). We write in page 3: “In a recent meta-analysis examining the efficacy of n-3 supplements (EPA+DHA) in the secondary prevention of coronary heart disease (CHD), authors analyzed 13 RCTs involving 20,485 patients with a history of CHD and concluded that n-3 supplements did not reduce CHD mortality, all-cause mortality and the risk of overall CVD complications (24).”

This is a fact, not an opinion, and we think that the quoted study “conforms to scientific standards”.

4- “In JELIS, a significant treatment effect was seen, as was demonstrated very convincingly, e.g. in the Kaplan Meier curves. Clearly, however, the overall risk, especially for fatal events in JELIS was low, lower than in comparable Western populations (e.g. in HOPE or EUROPA). This should be interpreted as an effect of the high background intake of omega-3 fatty acids in Japan, rather than as eicosapentaenoic acid being ineffective.”

We do not disagree with the comments of Dr von Schacky about JELIS. There is no doubt that the JELIS investigators reported a significant effect (p=0.01) of n-3 on the primary composite endpoint.

Our own comments are not in contradiction with Dr von Schacky’s statement. As for the meta-analysis cited above (ref 24), we are focusing our analysis on secondary prevention and in JELIS, authors actually give specific data regarding secondary prevention with n-3.

To underline the point, we have slightly modified the sentence in the Abstract of the revised manuscript: “Finally, in two large RCTs (JELIS and GISSI-HF) combining a statin with n-3 no significant protection was observed among patients with ischemic heart disease.”

Finally, as discussed in the present manuscript (page 8), JELIS had major design issues: for unknown reasons, the trial is not double-blinded and not placebo-controlled. In addition, the primary endpoint is a “composite endpoint” including disparate cardiovascular complications: hard endpoint (such as cardiac death) and very weak endpoint such as angioplasty (which is not really a complication but a therapeutic decision).

The use of “composite endpoint” is potentially associated with bias; see for instance: “Problems with use of composite end points in cardiovascular trials: systematic review of randomized controlled trials. BMJ 2007 Apr 14;334(7597):786” or “Composite outcomes can distort the nature and magnitude of treatment benefits in clinical trials. Ann Intern Med. 2009 Apr 21;150(8):566-7”]. All these design issues make the results of JELIS (and of many other trials, such as JUPITER discussed in our answers to reviewer 3) questionable.
Thus, we have done slight modifications in the paragraph regarding JELIS in the revised manuscript underlining the design issues and why we are focusing on patients in secondary prevention.

5- “That statins have no effect in GISSI-HF cannot be called a surprise. It had previously been demonstrated in the CORONA trial (N Engl J Med. 2007;357:2248-61). Also, if looking at the causes of death in both CORONA and GISSI-HF, almost no deaths fell into the category that statins can prevent, e.g. a myocardial infarction. This has nothing to do with an inhibition of the action of omega-3’s by statins.”

Below is a scan of the Table 2 showing the main results in the CORONA article cited by Dr von Schacky. We can see (arrows) that many nonfatal stroke and myocardial infarction occurred in both groups; also there were many fatal and nonfatal coronary events (second arrow, n=588 and 554) and among the deaths from cardiovascular causes (third arrow) 283 and 272 occurred in coronary events against only 191 and 193 deaths due to worsening heart failure.

In other words and in accordance with the cholesterol-statin theory, the risk of most of these ischemic events should have been reduced by the statin treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (N = 2497)</th>
<th>Rosuvastatin (N = 2514)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Patients</td>
<td>Event Rate</td>
</tr>
<tr>
<td>Primary outcome</td>
<td>732</td>
<td>12.3</td>
</tr>
<tr>
<td>Death from cardiovascular causes</td>
<td>487</td>
<td>488</td>
</tr>
<tr>
<td>Nonfatal myocardial infarction</td>
<td>141</td>
<td>115</td>
</tr>
<tr>
<td>Nonfatal stroke</td>
<td>104</td>
<td>89</td>
</tr>
<tr>
<td>Secondary outcome</td>
<td>759</td>
<td>12.2</td>
</tr>
<tr>
<td>Death from any cause†</td>
<td>588</td>
<td>10.0</td>
</tr>
<tr>
<td>Any coronary event‡</td>
<td>588</td>
<td>10.0</td>
</tr>
<tr>
<td>Fatal event</td>
<td>593</td>
<td>9.6</td>
</tr>
<tr>
<td>Death from cardiovascular causes§</td>
<td>327</td>
<td>5.3</td>
</tr>
<tr>
<td>Sudden death§</td>
<td>284</td>
<td>284</td>
</tr>
<tr>
<td>In primary outcome</td>
<td>283</td>
<td>272</td>
</tr>
<tr>
<td>In coronary events</td>
<td>191</td>
<td>3.1</td>
</tr>
<tr>
<td>Worsening heart failure</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Unexpectedly the statin (rosuvastatin) failed in CORONA!

We consider that the statement made by Dr von Schacky about CORONA is therefore not based on actual facts. It should not be taken in account.

If the statins are not effective to prevent ischemic heart disease complications and stroke in these high-risk patients, why would physicians prescribe them to low-risk patients?

Let’s face the obvious: the large numbers of ischemic complications in CORONA were not unexpected – neither the sponsors nor the investigators were naive enough to launch a very expensive trial without the hope that the statin will be effective – because 100% of the recruited patients were survivors of a previous infarction and thus expected to be at high risk of recurrence, the best situation in theory to prescribe a statin!
The fact that they also had various degrees of post-infarction left ventricular dysfunction and symptoms of chronic heart failure does not change the problem, as perfectly understood by the sponsor when launching the trial.

As a matter of fact, when looking at the effects of rosuvastatin in function of the severity of chronic heart failure, we see again no difference between the groups (Table below from the CORONA report): those with mild heart failure (NYHA class II) also had no reduction of the primary endpoint: 219 vs. 217 events in the placebo group.

Let’s go one step further. CORONA was the first trial testing statins in secondary prevention since the implementation of the New Clinical Trial Regulation. It proved negative despite a striking reduction of cholesterol (and CRP), thus raising one major question: did the previous “positive” trials with statins in secondary prevention conform to scientific standards?

As discussed in our article about the landmark 4S trial in secondary prevention (pages 15-16), we think that this is very doubtful.

GISSI-HF is a different question because only 50% of the patients were survivors of a previous infarction, and thus in secondary prevention. However, regarding the occurrence (or recurrence) of ischemic events (myocardial infarction and stroke), the same trends were observed in GISSI-HF as in CORONA with a total lack of effect of the statin regarding the ischemic events expected to be prevented by the statin.

We respectfully strongly disagree with Dr von Schacky. The lack of effect of the statin drug in CORONA and GISSI-HF came indeed as a very bad surprise for the sponsor as well as for the investigators of both trials.

6- The relation between red cell arachidonic acid and coronary events is J-shaped (Am Heart J. 2008;156:1117-23). The higher arachidonic acid, the higher the risk is not supported by these data”

The authors – of the article in the American Heart Journal cited by Dr von Schacky – write in their conclusion section: “The association of blood cell arachidonic acid content with ACS was U-shaped with levels in the highest quartile being robustly associated with odds for ACS case status”.

The statement by Dr von Schacky is thus not concordant to facts.

It is noteworthy that the cited study is a case-control study with major limitations. We prefer discussing data from controlled trials in humans, as we do in our present analysis; and we do confirm that “n-3 and n-6 fatty acids are in competition through various pathways involved in the development and complications of CHD” (first paragraph page 10). We have however decided to cite the study by Block in the Am Heart J (now ref 44) cited by Dr von Schacky.

7- “Most experts and opinion-makers do have major conflicts of interest and strong links with the pharmaceutical industry and/or various sponsors does not help shedding light on the true statin benefit/risk ratio in the everyday medicine” This is general mud-slinging and not scholarly writing.”

This is not mudslinging or insinuation, this is reality. We are stating a fact, not qualifying as unethical or dishonest, as it is apparently interpreted by Dr von Schacky.

We are not saying it is “bad” or “unethical”, we are stating facts.

The main question is: do conflicts of interest and/or lack of independence of the investigators prevent getting the right information for the care of our patients?

If the answer is no, why do Health Authorities and Editors ask we disclose our conflicts of interest?

Regarding “scholarly writing”, we can’t see the problem here. Stating that the existence of conflicts of interest “does not help shedding light on the true statin benefit/risk ratio in everyday medicine” is not a sacrilege statement …

8- “Statins have limited efficiency, and are no panacea. After statins were established in cardiovascular prevention, it became unethical to perform placebo-controlled studies with statins in that area. Industry then tried to push the envelope by performing trials in populations less prone to the effects of statins, e.g. congestive heart failure, chronic kidney failure asf. Not surprisingly, the results of these trials were neutral.”

This admittedly respectable opinion is a very personal way of looking at things.

However, beyond personal “opinions”, there are facts and as scientists we have to deal with facts: for instance, the fact that statins had no effect in CORONA and GISSI-HF – two recent placebo-controlled trials in secondary prevention – as discussed above.

Another fact is the lack of effect of statins in high-risk patients with diabetes and/or chronic kidney failure.

It is noteworthy that before the publication of all these recent negative trials, some experts had claimed – on the basis of questionable secondary analyses and meta-analyses pooling subgroup data from past trials – that statins are extremely effective in patients with chronic kidney failure or diabetes. At that time it was well accepted by most (not all) scientists and experts that statins are extremely effective in these high-risk patients in accordance with the famous claim “higher the risk higher the benefits of cholesterol-lowering” which appears to be totally wrong.

Also the fact that, as written in our revised article (page 13), “since the Vioxx/Celebrex debacle, the implementation of the new Clinical Trial Regulations and the Good Clinical Practice Directive 2005/28/EC, there have been fundamental changes in the conduct and reports of RCTs. Inspections by Health Authorities now concern study sites, laboratories, sponsors and contract research organizations. Clearly the prevalence of bias, spin and misreporting in RCTs has significantly decreased although confusion and controversies still exist
regarding the quality of many studies, as well as the safety and real benefits of many marketed products … since the implementation of the new Clinical Trial Regulations, all the RCTs testing the effects of statins in patients at high risk of CVD and expected to get large benefits of cholesterol-lowering – patients with post-infarct left ventricular dysfunction (39,40), chronic kidney failure or diabetes – were either negative or sometimes obviously flawed or misinterpreted …”

We admit that Dr von Schacky does not agree with this “interpretation” of the facts and we fully respect his opinion.

In the present analysis, however, we are not giving “personal” opinions. When we say that some trials are flawed or misinterpreted, not only we cite many eminent scientists – from ref 116 to ref 127, and these 12 references are only a short extract of a vast literature discussing the validity of many trials – but also we show how it has been done. In our own articles (ref 130,133,134) – which have been published after strict reviewing by independent referees and editors of different journals – we show the techniques used to flaw the results of some trials, for instance with JUPITER (see below in our answers to reviewer 3).

May we suggest Dr von Schacky to also read again our most recent articles on statins, for instance our systematic review (ref 130) about statins in patients with type 2 diabetes [in Rev Recent Clin Trials 2012, 7:150-7]?

9- “The verbiage used on page 12 insinuates a conspiracy of enormous size, something that would include unethical and unscientific behavior by large numbers of persons involved in clinical trials. If there is direct evidence for such a conspiracy, it should be brought forward. Insinuating a conspiracy by relying only on plausibility is not acceptable.”

We disagree with the term "verbiage". On the other hand, we totally agree that “Insinuating a conspiracy by relying only on plausibility” is not acceptable, reason for which we actually don't.

Not only do we not see any conspiracy, we do not even suggest there is one!

What we do state is that there are large amounts of “poor science”, in particular in the statin trials and statin meta-analyses – but also in many n-3 studies – as discussed all along our answers to the referees.

What we do state is that until the Vioxx/Celebrex tragedy, the Health Authorities have been extraordinary soft on these medical and scientific issues both in the USA and in Europe.

We do believe that the present multiple controversies (glitazones, rimonabant, anticoagulants, antiplatelet drugs, and so on) result from a mix of “poor science”, mediocre expertise and Authorities’ laxness, not from any conspiracy!

10- “Minor comments: A negative trial is a trial with a negative result, i.e. intervention worse than comparator. Recent trials with omega-3’s were neutral.”

We disagree. And this is not a minor point as it suggests a major misunderstanding of modern clinical sciences! In our view, a trial is an experiment in humans designed to test a specific (a priori calculated) question: for instance, is the tested drug better than the placebo?

Contrary to the idea of Dr von Schacky, we never test whether a drug is worse than the placebo; it would be unethical. It means there is never any calculated a priori hypothesis to test whether a treatment is toxic, or worse than the placebo …
At the end of any trial, we must answer the question: are the results with the drug (significantly) different from those with the placebo?

There are only two possible answers: yes and the trial is said “positive” or no and the trial is said “negative” meaning that investigators have not been able to confirm their a priori hypothesis.

Because it has been consensually decided to use a rigid frontier \([p<0.05]\) between the yes and the no, there is no “neutral” trial and there is no “maybe”. Dura Lex, sed Lex!

Sometimes, this “dura lex” looks like too rigid because one trial would be negative with a \(p=0.05001\) whereas another trial would be positive with a \(p=0.04999\).

However, once our students have understood the “a priori hypothesis principle”, they never continue to think that the results of a trial are “neutral”.

Sometimes however (as discussed above with Dr Billman), it is right to say in a discussion (interpretation) about a study that the results are “borderline” significant or not significant …

**REVIEWER 3**

1. “The authors have written a review examining the effects of \(n\)-3 PUFA and statins and their interactions when used for the prevention of cardiovascular disease. The paper includes 4 major interesting and sometimes controversial observations. 1) statins appear to inhibit the benefits of \(n\)-3 PUFA, 2) The benefit of \(n\)-3 PUFA supplementation is minimized in patients with greater baseline \(n\)-3 PUFA consumption, 3) The beneficial effects of statins have not been evident in recent trials and the clinical enthusiasm for the use of statins is not justified given their true efficacy and side effects, 4) Early studies showing the beneficial effects of statins may have been significantly influenced by investigator bias. The first 2 points are well supported by the presented evidence and are important observations supported by original synthesis of the existing data.

We thank Dr Raitt, for these kind comments.

We greatly appreciate the comment that the first two points are “well supported by the presented evidence”.

We will do our best in the next paragraphs to support the statin issue.

Before discussing each remark of Dr Raitt, we would like underlining that among the many flawed trials we have analyzed, the “investigator bias” was only one of the possible explanations; there are some others as discussed below.

2. The third assertion is controversial and in my opinion is not as well supported as it should be. It is unclear in what clinical situations the author is objecting to the data for the beneficial effects of statins: primary prevention, secondary prevention, or both. Since the use of fish oil in the context of this paper is in patients with established heart disease then the relevant indication to question would be secondary prevention. The cited studies are primary prevention.

We disagree: the cited studies are not primary prevention.

For instance, if we restrict the present comment to the trials testing rosuvastatin – because all the trials testing that specific drug have been published after the implementation of the new Clinical Trial Regulation, and this is a critical issue – there are 4 placebo-controlled trials: CORONA, GISSI-HF, AURORA and JUPITER.
As shown in our answers to Dr von Schacky, 100% of the patients enrolled in CORONA are survivors of a recent myocardial infarction (secondary prevention); and 50% in GISSI-HF.

In AURORA (chronic kidney failure patients, ref 112), more than 50% of the patients had some cardiovascular diseases (see the table below from the AURORA article) in addition to their kidney problem and some other risk factors.

Finally, in JUPITER, no patient was considered as having established ischemic heart disease (primary prevention), but the investigators do insist on the fact that these patients are at high risk (thus different from the general population) because of a metabolic syndrome, or some aspects of the syndrome (hypertension), and all had (inclusion criteria) a chronic inflammatory syndrome (high CRP).

In another recent negative trial – the German 4D trial testing atorvastatin this time in diabetics (ref 128) – a high proportion of the patients had overt cardiovascular disease when entering the trial (see below the table from the article).
In another negative trial testing atorvastatin in diabetics (the ASPEN trial, ref 129), also a large proportion of patients had a previous myocardial infarction and were in secondary prevention.

Thus, the cited negative studies were not in primary prevention.

3- “In fact, I think the argument regarding recent studies being negative is in large part because recent studies were not secondary prevention studies.”

As explained above, this statement is inaccurate.

4- Furthermore, though the author reverences one of his own papers on the topic there is no mention of the Jupiter study which was published in the New England Journal of Medicine in 2008 and was a strongly positive primary prevention trial with more patients than the negative studies reviewed in the paper and was published around the same time.”

Dr Raitt is likely referring to our article in World Rev Nutr Diet (ref 134) published in 2009 but submitted in 2008 when the JUPITER report was not published yet.

We have later on fully discussed the JUPITER trial in our article (ref 133) in the Archives of Internal Medicine published in 2010.

5- “It is an interesting, important, and highly controversial assertion that statin study results have been affected by biased investigators. Given the implications of such an assertion I believe it should be rigorously supported in the text. Pointing out the possible appearance of conflict of interest is important but not an adequate support for such a sweeping assertion. The authors do go into more detail in other papers they have written and referenced in this paper. In my mind such a reference is not adequate to support the assertion.”

The present article was not written to show “investigator (or sponsor) bias” in statin trials. It would take a book. Also, we do not simply “point out the possible appearance of conflicts of interest” as written by Dr Raitt, we cite several articles, including from our group, showing bias and flaws. The question is: should we repeat in the present review the arguments and facts developed in each cited article?

6- “In fact, when I read the referenced critique of the Jupiter trial I was not convinced by the authors’ arguments. Others may agree with them but I think if one is going to make such strong iconoclastic assertions the details supporting it should be in this paper.”

There are many design and trial conduct issues in JUPITER and we cannot discuss all. We are going to review some of them.

One of them is that the trial was prematurely terminated: after less than two years of follow-up in average. This was a major modification in the trial and there is no need to be a great statistician to understand that even a two-year trial is really very short in the context of primary prevention which is usually considered by most physicians as a long-term preventive strategy. Thus, it was not a futile decision, all the more as many experts claim that earlier termination of a trial is a major cause of bias; please see ref 131 as an example.

The main question is whether the reasons given by the investigators for early termination in JUPITER were legitimate or minimally justified and a source of bias or not.

The investigators claimed that they had to stop the trial because it would have been unethical to continue to give a placebo in the control group. See the press release published by the sponsor during the meeting of the American College of Cardiology in March 2008 in the
following document: JUPITER halted: rosuvastatin significantly reduces cardiovascular morbidity and mortality. MedscapeWeb site. http://medscapemobile.com/viewarticle/572270. Thus, the main justification given by the sponsor and the investigators was an “unequivocal reduction of cardiovascular mortality in JUPITER …”

This was the first version of the story.

Beyond the (debatable) scientific validity of such an assertion, it is critical to verify whether cardiovascular mortality – by far the best hard endpoint we have in cardiovascular epidemiology – was actually reduced. The scan below shows the results of JUPITER as they were presented in the New Engl J Med in November 2008.

Table 3. Outcomes According to Study Group.

<table>
<thead>
<tr>
<th>End Point</th>
<th>Rosuvastatin (N=8501)</th>
<th>Placebo (N=8501)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Patients</td>
<td>Rate per 100 person-yr</td>
<td>Rate per 100 person-yr</td>
</tr>
<tr>
<td>Primary end point</td>
<td>142</td>
<td>251</td>
</tr>
<tr>
<td>Nonfatal myocardial infarction</td>
<td>22</td>
<td>62</td>
</tr>
<tr>
<td>Any myocardial infarction</td>
<td>31</td>
<td>68</td>
</tr>
<tr>
<td>Nonfatal stroke</td>
<td>30</td>
<td>58</td>
</tr>
<tr>
<td>Any stroke</td>
<td>33</td>
<td>64</td>
</tr>
<tr>
<td>Arterial revascularization</td>
<td>71</td>
<td>131</td>
</tr>
<tr>
<td>Hospitalization for unstable angina</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>Arterial revascularization or hospitalisation</td>
<td>76</td>
<td>143</td>
</tr>
<tr>
<td>Myocardial infarction, stroke, or confirmed death from cardiovascular causes</td>
<td>83</td>
<td>157</td>
</tr>
<tr>
<td>Death from any cause</td>
<td>190</td>
<td>235</td>
</tr>
<tr>
<td>Death on known date</td>
<td>198</td>
<td>247</td>
</tr>
</tbody>
</table>

Curiously cardiovascular mortality is not reported separately in this second version of JUPITER results.

We can of course calculate fatal infarction and fatal stroke – the sum of which usually represents the vast majority of cardiovascular deaths in any population – since the authors give the numbers for nonfatal infarction, any infarction, nonfatal stroke and any stroke. By making the subtractions (“any” minus “nonfatal” for infarction and stroke), we obtain 12 deaths in each group. In other words, there was no difference between the two groups. Moreover the numbers were very small indicating that it was a big mistake to stop the trial. Discussing whether this was an “investigator bias” or a “sponsor bias” is pointless!

Still more curiously, the authors proposed a third version of cardiovascular mortality in a memorandum published by the FDA in November 2009. See below.
There were 37 and 29 cardiovascular deaths in this third version of JUPITER.

A fourth version of cardiovascular mortality in JUPITER was proposed in December 2009 that anyone can consult in the FDA document on page 31 of the following document: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/EndocrinologicandMetabolicDrugsAdvisoryCommittee/UCM194918.pdf.

In that fourth version, there were 44 cardiovascular deaths in the placebo group and 35 in the statin group; and for the first time the sponsor reported 18 sudden cardiac deaths in the placebo group and 9 in the statin group. Also, there were 10 “other” cardiovascular deaths in the placebo group and 12 in the statin group but we do not know exactly how these patients died.

The “sudden” apparition of 27 sudden cardiac deaths in that fourth version is quite shocking! Readers of the main article in the New England Journal of Medicine – the medical article upon which most physicians use to base their prescriptions – would have been interested to know that.

There is finally a fifth version, published by leading author P. Ridker in the American Journal of Cardiology in 2010 in which the following figures are given:

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Rosuvastatin 20 mg N=8901</th>
<th>Placebo N=8901</th>
</tr>
</thead>
<tbody>
<tr>
<td>First MACE</td>
<td>142</td>
<td>252</td>
</tr>
<tr>
<td>Cardiovascular death</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>Non-fatal MI</td>
<td>21</td>
<td>61</td>
</tr>
<tr>
<td>Non-fatal stroke</td>
<td>30</td>
<td>51</td>
</tr>
<tr>
<td>Hospitalized unstable angina</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Arterial revascularation</td>
<td>41</td>
<td>70</td>
</tr>
</tbody>
</table>

In this report there are 35 and 43 “confirmed” cardiovascular deaths with, this time, 16 and 25 sudden deaths which is quite ambiguous …

Thus, we got five different versions of cardiovascular mortality in JUPITER …
If this is not poor science, we wonder what is!

It could be fair – as Dr Raitt does – to say there is no problem here – as all the versions report no significant difference – and we can trust the reported data and the investigators who reported them. This should not be our way of making medical science.

Once a dataset has been constituted (and frozen) at the end of the trial and before making statistics and before publishing it, it is not permitted to change it, of course … If we modify the dataset in order to prevent (or respond to) criticisms, this is called “data manipulation”!

In addition, having noticed the persistent lack of difference between the two groups (including in the fifth version) and therefore the lack of scientific, medical or ethical justifications for the premature termination of the trial, this variability in the results raises several questions: 1) which version is the good one? 2) How can the investigators explain this variability? 3) Who was really in charge of managing the raw data and the clinical files, and of validating and classifying the endpoints? 4) What were the role(s) of the sponsor on one side and of the investigators on the other side? 5) Which version should the physicians retain to guide their everyday practice?

To get an idea of the role of the sponsor, we have copied a paragraph of a recent article on JUPITER (below):

The major information here is that the study was conducted on the ground by employees of the sponsor who “collected the trial data and monitored the study sites”. It means that the academic statistician – a respectable person – only analyzed data supplied by the sponsor, including the clinical endpoint dataset …

It also suggests that the “independent” Committee working on the validation and classification of the endpoints only worked on data supplied by the sponsor …

Given the variability in the endpoint report, the major question for any scientist would be: how was the endpoint adjudication process in JUPITER? With another major question: were the raw data verified through an independent audit?

Central adjudication – assumed to be independent from the sponsor – is the conventional approach to maintain data integrity (using uniformed rules for assessment of clinical endpoints in the different recruiting sites) and blinding.
In JUPITER, it seems that the adjudication process (validation and classification of the endpoints) was not independent of the sponsor since the sponsor itself collected the data.

This is a critical issue because recent FDA secondary reviews (about the recent TRITON and PLATO trials) have shown major discrepancy between centrally adjudicated and site reported events which have strikingly influenced the overall results of these major clinical trials: from negative, they become positive showing that prasugrel is superior to clopidogrel, for instance. These FDA secondary reviews of the reported endpoints in multicentre “commercial” trials are one of the consequences of the New Clinical Trial Regulation following the Vioxx/Celebrex tragedy. It is better than before, but far to be perfect because FDA officers still do not have access to the totality of the raw clinical data recorded in each Centre by the employees of the sponsor.


To get an idea regarding whether it was useful or not to make a secondary review of TRITON, please note that in the recent TRILOGY ACS trial, prasugrel appears to be not superior to clopidogrel this time (N Engl J Med 2012 Aug 25).

It is obvious for many experts that the Health Authorities should continue to demand independent audits to identify anomalies in the endpoint adjudication process and, for instance, discrepancy between the site-reported complications and the centrally-adjudicated complications. For a review of the issue, see for instance: Thromb Haemost. 2012 Jul 26;108(3) and Verheugt FW in Thromb Haemost. 2012 Jul 26;108(3).

In any case, it is now apparent that experts independent from the sponsor should have a look at the raw (hospital) data of each patient (including those who do not have any complication during the trial) before validating the dataset. External audit should be an obligation to re-introduce confidence regarding the validity of the dataset.

What about JUPITER and the five versions of cardiovascular mortality?

Apparently – we were unable to find any secondary review of JUPITER endpoint by the FDA officers – the Endocrine-Metabolic Division of FDA never challenged or disputed any data the sponsor presented on JUPITER. They simply accepted the sponsor JUPITER data as 100% true and without any mistake …

Our first conclusion regarding JUPITER is that we are facing “poor science”, mediocre expertise and tragic laxness of the Health Authorities if we use the same terminology as for our answer to Dr von Schacky regarding the great “conspiracy” …

In fact, the sole existence of five different versions of cardiovascular mortality should have motivated an independent audit of the raw data obtained in each site …

Given the variability of the five reports and the lack of independent audit, the next question is whether the JUPITER's results were clinically consistent. Actually, one way of indirectly check the existence of major bias in any trial is to look at external consistency.
For instance, does the ratio of cardiovascular to overall mortality calculated in JUPITER correspond to what should be expected, on the basis of what we know in various populations? Because of the variability of that item, such a discussion is useless … Which version would we use?

An alternative way to verify clinical consistency in JUPITER is to look at the case-fatality rate.

There is below a scan of a paragraph of our article in the *Archives of Internal Medicine* where we discuss that important issue. May we respectfully ask Dr Raitt to read it again?

Thus, clinical inconsistency in JUPITER is obvious and likely linked to the bias resulting from the premature termination and secondary “review” of the dataset by the investigators.

Finally, the overall mortality data in JUPITER should be critically examined. Actually, for any physician or cardiologist, the ultimate goal of preventive treatment is to increase life expectancy.

A significant effect on overall mortality in JUPITER has been often presented by the investigators, the sponsor and the media as a landmark result of the trial.
However, even the FDA officers have been extremely prudent with that assertion: below a scan of the paragraph regarding “total mortality” in the reports by the FDA statisticians.

Although Total Mortality (p=.021, HR=.80) was statistically significant at the .05 level when vital status data was used, it was neither a component of the MCE endpoint nor a secondary endpoint subject to Type I error control in the Statistical Analysis plan (SAP).

There were 198 deaths (2.2%) in the rosuvastatin arm and 247 deaths (2.8%) in the placebo arm. The Kaplan-Meier estimates at 4 years were 4.2% and 5.3% respectively, with an absolute risk difference of 1.1% and 95% confidence interval (0.3%, 1.9%).

Further inspection of the data shows that the Kaplan-Meier curves converge toward the end of the trial. At approximately 1,600 days (4.4 years), the Kaplan-Meier estimate of the absolute difference in risk of death is 0.7% in favor of rosvastain with a 95% confidence interval (-0.4%, 1.8%). Thus, it is not clear whether or not rosuvastatin confers a total mortality advantage compared to placebo even though the logrank test appears to detect the separation of the survival curves up to over 4 years.

There is below a scan of the survival curves published in November 2008 in the New England journal of Medicine, thus before the above report by FDA statisticians.
The fact that the two curves are converging beyond year 4 is clear and, as underlined by the FDA statisticians (above), it is not a detail.

This again underlines that it was a mistake to prematurely stop the trial, even if it is not stated by the statisticians whose remarks appear to be quite soft.

Surprisingly, in a subsequent article published in *Circulation*, a new version of the survival curves was proposed. The scans are copied below.

Curiously, the curves are no longer converging. We do not think that this is a fortuitous mistake.

For those who know how to interpret this kind of curves (see the above comments made by the FDA statisticians, for instance) and the associated numbers, it is a way of masking the reality of the data. But for the physicians who refer to the *Circulation* articles to get an opinion, what is it? Is it spin, misreporting, bias or data manipulation?

Neither the Editors of *Circulation* nor the FDA officers did apparently react.
Dr Raitt wrote: “if one is going to make such strong iconoclastic assertions the details supporting it should be in this paper”!

Given the present way of publishing scientific data, we do not think that such an enumeration of scientific “malpractice” would be “in line” with our present analysis of the data regarding the statin/n-3 interactions.

However, it will possible for any reader to read our present answers to the referee in the Pre-publication history section.

7- “Finally, I am not sure why the authors devote a large section of the paper to the efficacy and adverse effects of statins.”

As fairly admitted by the reviewer, it is quite clear that statins inhibit n-3.

There are two main possible explanations:

1) statins are so active that the residual morbidity/mortality rate in statin-treated patients cannot be reduced by any additional treatment – including n-3 – this is what we call a “dilution effect”;

2) statins actually interfere with the physiology of n-3 and prevent their protective effects.

It is therefore critical to show the many design issues in the statin trials – the recent and the past trials – and also the time concordance with the failure of n-3 trials.

If the protective effect of n-3 could have been reduced in relation with the correction of the n-3 deficiency in the populations, it remains that some patients are still more or less deficient – in particular in secondary prevention – and could still benefit from n-3 supplementation. In that context, the lack of effect of n-3, in particular in the most recent and well-conducted trials such as the Alpha Omega trial (ref 23) and the ORIGIN trial (ref 148), could be explained by the large use of statins in these studies.

In support of that explanation, it is important to show that the protective effects of n-3 are inhibited even when the statins are not effective; and that biologic interactions between statins and n-3 have been clearly identified.

8- “I presume it is because they feel statins inhibit the benefits of n-3 PUFA and therefore clinicians should choose one or the other and that the authors think that n-3 PUFA is the better choice. I think it would be a no less important paper if the authors focused on the question of an interaction between the two ”medications”. I do not think this point is proven. First the recent data for n-3 PUFA is not great even if one takes into account the potential confounder of statins and second even in light of the authors concerns about the statin data, the data for statins in secondary prevention of CAD is much stronger than that for n-3 PUFA.

The way of reasoning of Dr Raitt is elegant but based on one basic assumption which has not been confirmed in all recent trials: the fact that statins are effective in secondary prevention. If statins are not protective – and this is what we have seen in recent trials, as shown above – then the reasoning of Dr Raitt does not work anymore.

In any case, the job of the scientists is to propose a unifying theory which could encompass the whole picture. This is what we are proposing.

9- I suggest that this section be removed as I do not think there is room in the paper to support the assertions about the statin data and I do not think that the authors will have the data to effectively
Bias and flaws in statin trials are only a small part of the question regarding the validity of the results of “commercial” trials in general.

We are sorry to say that the Vioxx/Celebrex tragedy was not “a cloud in a blue sky”. Beyond the statin issue, bias and flaws have been reported in many commercial trials, even recently as shown (see above) about the PLATO and TRITON trials. May we suggest the reviewer to consult the references 116 to 127 which indicate that we are not the sole scientists to think that we are facing a big problem of credibility about commercial trials?

We – and many others (see for instance JAMA January 4, 2012, page 37-38) – actually think that the results of many commercials trials have been flawed, in particular those testing the statins. We do not think it is a “strong iconoclastic assertion”, as said by Dr Raitt. And we not think there is any conspiracy, as discussed in our answers to Dr von Schacky.

The question of the effects of statins in secondary or primary prevention is a major issue. Many experts think that statins are protective in secondary prevention but not in primary prevention. However, a myocardial infarction or a stroke in primary prevention results from the same pathological process as an infarction or a stroke in secondary prevention. The only difference between primary and secondary prevention is the level of likelihood in the tested populations: in patients with a prior infarction, the risk is higher than in healthy people without previous heart attack. The mechanism (thrombotic obstruction) is the same but, given the very different probability of observing such complication in the two populations, the sample size and duration of follow-up should be adapted. We needed thousands of healthy people to demonstrate the antithrombotic effect of any treatment but only hundreds survivors of infarction …

**Back to statins:** if we admit they are not protective in primary prevention (many experts agree), there is no scientific reason to still believe they are protective in secondary prevention. We could also say that if there is any effect in either primary or secondary prevention, it is very small – therefore not clinically important – and this does not justify their massive use in both primary and secondary prevention. In support of that view, all the recent statin trials have been negative – including in secondary prevention and in high-risk patients – or obviously flawed as exemplified with JUPITER (see above).

10- “It might be reasonable to suggest that in situations where the utility of statins is less clear and that of n-3 PUFA is more apparent that statins should not be used but this will be a somewhat unusual situation.”

It might be reasonable to review the whole statin story – as well as the whole n-3 supplement story – and this should be done since the beginning as it is now a critical issue. Actually, on the basis of the most recent trials (as discussed above), we do not see “situations where the utility of statins is clear”, as written by Dr Raitt.

This review from the beginning may help to define how to use n-3 supplements in the present days. In our opinion, n-3 supplements can be useful in certain situations, but in primary prevention, there are many other (nutritional) ways of getting the n-3 we need.

11- “Major Compulsory Revisions: 1) Since the presented data for n-3 PUFA is for secondary prevention please limit the review of statin efficacy to secondary prevention.”
As explained above, the whole statin story must be reviewed.

12- “2) If the authors decide to continue to include the discussion of potential bias and ethical misconduct in statin studies they will need to present more original specific examples of studies in which there is reason to believe there is bias and explain why.”

It is not our role discussing any ethical misconduct. Our goal is just to show that there are many biases that explain why trials are flawed.