Author's response to reviews

Title: The effects of EPA and DHA enriched fish oil on nutritional and immunological markers of treatment naïve breast cancer patients: A randomized double-blind controlled trial

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Reviewer #1:

Overall, the manuscript is clear, concise and very well written reporting novel findings in an area of importance.

1. It would have been of interest to measure fatty acid uptake into PBMCs given that incorporation into leukocyte membrane are proposed to be essential for immunomodulation.

   We agree with your suggestion. Unfortunately we did not have enough blood sample for this analysis to be taken.

2. Please add reference to Tables 1 and 2 at first mention in the text (line 230-242).

   The results in these lines (baseline characteristics) were only described in the text form. Therefore, there is no table associated to these results.

3. Line 263: An increase in plasma n-6:n-3 ratio is reported in the FG, however it should report a DECREASE.
Yes, thank you for your important observation. It was corrected. Please, see page 11, line 266.

4. Line 330: Please replace the word 'NO' with 'NOT' in the context of 'not significant'.

Thank you for the observation. It was corrected. Please, see page 14, line 337.

5. Figure 2: Please provide a legend to the symbol used in 2A and 2B.

Thank you for the observation. We have detailed the information in the legend for the figures 2A and 2B.

Reviewer #2:

This paper tested 4 weeks of fish oil supplementation vs placebo on markers of inflammation and omega 3 incorporation in patients newly diagnosed with breast cancer. This is an area with scare information and it is good to see a well-controlled study being conducted. There are a number of points to be addressed for clarification

1. P3, line 77- in the systematic review (ref 12) what time frame of supplementation was associated with a reduced risk?

The systematic review (SR) (ref 12) was based on prospective cohort studies that met the inclusion criteria and the mean follow-up period of the studies ranged from 4.3 to 20 years. The studies were not intervention trials and, thus, the usual dietary intake of n-3 fatty acids were considered, instead of supplements. Please, see page 3, lines 76-77 of the manuscript, where we have made short improvement/correction regarding information from the SR.

2. P3, line 72-73 remove "in consequence"

Thank you for your observation. It was removed.

3. P4- check ref 14 e

Thank you for your observation. In fact, in the study by Faber et al, CRP did not show significant difference between the groups. We have corrected the citations in the text accordingly. Please, see page 4, line 82.

4. P4, line 81 is improved body weight an increase or decrease in this instance?
Patients increased their body weight. We made the change in the manuscript accordingly. Please, see page 4, line 81.

5. P6, line 150 - how was BMI classified? - a reference should be provided or further details

We used the World Health Organization classification for BMI and we have added the reference. Please, see page 6, lines 150-151.

6. P6, line 155 - does NutWin have multiple databases to choose from? - please specify

NutWin uses the USDA food composition database for nutrient calculation. This information was added in the text. Please, see page 7, lines 156-157.

7. P9, given the high dropout was the study sufficiently powered? A retrospective power calculation would be helpful

Power calculation was not presented because primary end points of this study (nutritional status/body weight) have not been reported in breast cancer patients receiving n-3 fatty acids prior to treatment. However, we considered the results of Bougnoux et al (2009) study, which assessed the effect of DHA in breast cancer patients during chemotherapy (that study found objective response rate to treatment in 44% of patients) and the existence of a (fictitious) control group that no more than 5% presented a positive response. Calculations determined that a sample of 18 subjects in each group would allow, with 80% power, confirmation that these differences (44% and 5%) are statistically significant at a 5% significance level. However, as we pointed in the last paragraph of our discussion (page 14, lines 356-357), we are aware that our sample size was not sufficient for the primary end points of the study. We detected significant differences, that were subtle, for the secondary end points (immunological parameters) which we believe was an important new contribution of our study.

8. P10 - need to report on lipids and glucose results - where are these data reported? They should be in text or a table

The results on these parameters were reported in the last paragraph of the sub-section “baseline characteristics” (page 10, lines 243-244) and “intervention effects” (page 11, lines 281-281). No differences were observed within or between groups and therefore the values were not reported.

9. P11, line 271 - as the CRP change was not significant this should be stated rather than close to significant

We changed the wording accordingly.
10. P11, line 273-275 - what changes occurred in CD8+ in the control group

We did not observe changes in CD8 counts in the control group. This information was added in the results section. Please, see page 11, line 278.

11. P12, check grammar lines 291-295

Thank you. We have reviewed the sentences.

12. P13, lines 314-325 - this section focuses on changes in CD4+ but doesn’t comment on CD8+ or the ratio- what are the implications of only CD4+ T lymphocytes changing

We thank you for the observation. We have modified the paragraph in page 13, lines 318-332 considering not only the CD4+ but also the CD8+ and the ratio. The new paragraph is: “Low peripheral blood CD4+ counts [5,6] have been observed even in the early stages of breast cancer patients. Whereas the number of circulating T CD4+ lymphocytes decreased in the placebo group, which is in line with the suppressor substances produced by tumor cells as its immune escape mechanisms, the maintenance of the number of T CD4+ lymphocytes in the n-3 fatty acid treated group may have been due to the proliferative effect of fatty acids on lymphocyte functions [2]. In patients of the placebo group, although the number of TCD8+ lymphocyte did not change, the possibility that the lower number of TCD4+ lymphocytes might have impaired proliferative capacity of the TCD8+ cells cannot be ruled out, because helper function of TCD4+ lymphocytes is required to full activation of TCD8+ cells [28]. As the number of TCD4+ and TCD8+ lymphocytes and its ratio remained stable in the fish oil treated group, the results of our study could suggest a positive effect of fish oil supplement in the adaptive immunity. Surgery is the mainstay of treatment of these patients and this procedure induces substantial immunomodulation, with pro-inflammatory response and leukocytosis [29]. Thus, a balanced adaptive immune response may help prevent postsurgery immunosupression and risks such as tumor dissemination into the circulation [30].”

13. P13. Line 331 states the previous study ref 14 saw a change in PGE2 but this study did not- would you expect a change in 4 weeks?

Yes, because incorporation of fatty acids and changes in peripheral blood PGE2 levels were seen after few days of intervention (7-day intervention trial with cancer patients under radiotherapy, ref 14). Although that study supplemented higher amounts of n-3 fatty acids than our study, we expected that 4 weeks of supplementation could show a cumulative response.

14. I suggest removing the abbreviations in table 1

Thank you for the suggestion. It was removed.