Author’s response to reviews

Title: Omega-3 intake is associated with attenuated inflammatory response and cardiac remodeling after myocardial infarction

Authors:
Alessandra Campos-Staffico (ale_menezes@me.com)
Ana Costa (anapaularez@hotmail.com)
Luiz Carvalho (luissergiofc@gmail.com)
Filipe Moura (filipe_a_moura@hotmail.com)
Simone Santos (sns2003@uol.com.br)
Otavio Coelho-Filho (tavicocoelho@gmail.com)
Wilson Nadruz (wilnj@fcm.unicamp.br)
José Quinaglia e Silva (quinagliaesilva@yahoo.com.br)
Andrei Sposito (andreisposito@gmail.com)

Version: 1 Date: 02 Apr 2019

Author’s response to reviews:

Response to comments:

Reviewer 1

Q.1. Line-58: Authors found that "The intake of ω-3 below the median (<1.7 g/day) was associated with a short-term increase in hs-C-reactive protein [OR:1.77 (1.17-2.66); p=0.007], Interleukin-2 [OR:1.95 (1.01-3.75); p=0.045], brain-type natriuretic peptide [OR:2.40 (1.21-4.75); p=0.012], left-ventricle end-diastolic volume [OR:11.72 (1.16-118.04)]; p=0.037] and decreases in left-ventricle ejection fraction [OR:4.36 (1.16-16.33); p=0.029] after adjustment for covariates." This doesn't mean that the intake of ω-3 above the median (>1.7 g/day) is associated with changes in acute inflammatory biomarkers, left ventricular remodeling, etc. in the opposite direction. Therefore, authors' conclusion that "These findings suggest that an elevated daily consumption of ω-3 may mitigate outcome-determining changes after STEMI, such as acute inflammatory response and late left ventricular remodeling" is not consistent with their finding. Authors should either present their findings for consumption of ω-3 above median (and conclude accordingly) or conclude that consumption of ω-3 below median may result in worse acute inflammatory and left ventricular remodeling outcomes.
We agree with the Reviewer and we changed the conclusion to incorporate his/her suggestion.

Q.2. Line-82: Revise the phrase "at the time of the coronary event" to clarify that you are referring to the period prior to the event.

The abovementioned sentence was appropriately reviewed, and replaced by “…ω-3 intake prior the onset of the coronary event.”

Q.3. Line-96: Please correct this: "It was not included patients."

The phrase was reviewed, and replaced by “Participants with new or presumed left bundle branch block, development of pathological Q waves without ST-elevation recording or exclusively with imaging evidence of MI were excluded.”

Q.4. Line-123: Authors need to provide citation or database for the photographic record for dietary surveys. Recall bias can be high when you ask MI patients about three months dietary history, frequency, and amounts, so you need to admit it clearly in limitations.

Recall bias was properly inserted as limitation of this study.

Q.5. Methods: Authors don't have to describe clinical and biochemical measurements, echocardiography, and CMRI in detail, if these are described in the original study. You can summarize them and provide reference to the original source for specifics. However, more relevant measurements, such as ω-3 and other nutrient intakes should be provided in detail.

All pointed topics were reviewed and duly summarized with respectively references cited. In contrast, nutritional methods were provided in details.

Q.6. Line-147: Word "data" is plural. Also it should be corrected as "normally distributed".

Both corrections were made in the manuscript.

Q.7. Line-150: "comparison" or association?

We corrected as indicated by the Reviewer.

Q.8. Line-155: The sentence starting with "Multivariable binary logistic regression was used to" is not clear. Are predictor variables continuous or did you make them binary? Also, why did you
use binary logistic regression, but not repeated measures ANOVA, considering that you have multiple time-points?

We thank the reviewer for pointing out to us the lack of clarity of these sentences. The text has been rewritten to improve clarity. What we wanted to mention is that we used binary logistic regression, thus dichotomizing the dependent variable, in this case was the ingestion of omega-3. These independent variables were categorized into below or above their respective medians to bypass the non-normal distribution and as a strategy to level their effects sizes, allowing direct comparability between variables. We preferred to use the regular ANCOVA to adjust to the confusional effect of the regression to the mean, which in this case can become an important bias. Thus, the comparison of the change of the CRP, BNP, IL-2 between admission and the fifth day were adjusted by respective baseline values and by independent variables selected using bootstrapping based on t test analysis: Age, gender, diabetes mellitus, hypertension, coronary reperfusion therapy, and use of ACE inhibitors / ARBs were selected as covariates.

Q.9. Line-227: This is not clear: "possibility of a play of chance due to the observational nature of this study".

We clarified and emphasized the possibility of selection bias due to observational design used in this study.

Q.10. Line-239: Please explain what you mean by "Indeed, we cannot rule out the possibility that individuals who consume more ω-3-rich foods also have undetected or unknown characteristics that may indirectly mediate the study findings"? If you are referring to confounding, describe them. If you are referring to recall bias or something else, state them explicitly.

We rewrite the text to make it clear that we are mentioning the possibility of unbalance between the study arms and consequently the risk of selection bias. “Firstly, as commented above, our findings must be considered as hypothesis generating due to the observational design and hence the impossibility to exclude selection bias. Randomized controlled trials (RCT) are required to exclude unbalance between the arms and the healthy cohort effect. However, consistency with RCT and mechanistic data deem this finding plausible.”

Q.11. Line-355: Although this is not RCT, show the time-points you assessed and omega-3 groups in figure-1.

Figure 1 was appropriately remodeled, and the suggested groups were inserted.

Reviewer 2

Introduction:
Q.1. The objectives of this study should be clarified.

Objectives were properly clarified at the end of introduction.

Method:

Q.2. Nutritional intervention method is appropriate but should also mention the recorders.

In order to clarify the sources used for nutritional intervention, new references were cited.

Q.3. For cardiac MRI protocol, the details of technique used (e.g. axis, slide thickness, field of view, etc.) should be presented.

In respect to the suggestion given by the Reviewer 1, all details about CMRI were suppressed in order to condense the text. However, a reference containing all required methods and details was cited in the manuscript.

Q.4. Coronary reperfusion therapy should be defined as PCI and/or fibrinolytic therapy. If both, the number of patients in each procedure should be presented. Time between MI onset and therapy and the result of the coronary reperfusion can be potential factors related to the study outcomes.

Coronary reperfusion therapy was duly defined in Clinical Evaluation section. All required data about time and number of patients underwent to each procedure are shown in Table 1. Indeed, coronary reperfusion therapy can be a potential confounder. For this reason, it was chosen as a covariate and took part in all used adjustment models.

Q.5. Please provide more details about GRACE score (for example, adding a reference).

A briefly explanation about GRACE risk score was provided followed by a reference.

Results:

Q.6. Even if history of smoking is not statistically significant in Table 1, it should be considered as a covariate concerning the previous evidence.

During the covariate selection, we had to be very precise, including all potential confounders, but without forget the risk of overfitting the regression models. Given that both groups had a balanced exposition to smoking, we decided not include it, once we already had 4 or more others covariates to compose the models. In order to adequately answer to the Reviewer, we repeated
modeling including smoking and using stepwise selection of variables and smoking was not maintained in the final model. We forced the insertion of smoking into the model and again there was no change in results. This information was added to the results.

Q.7. Why not include triglyceride or non-HDL cholesterol as covariate? AND Q.10. Table 2: Why were analyses not adjusted for age and sex? AND Q.11. Table 3: Why were the associations of omega-3 with Δhs-CRP, ΔIL-2 and ΔBNP not analyzed using Model 3?

We thank the reviewers for pointing out this subject. We repeated the logistic regression including all variables with significant difference in table 1 and selected the variables for the final model by stepwise. Thus, in table 3, we now consider model 1 non-adjusted analysis and model 2 fully adjusted analysis.

Q.8. Table1: Previous myocardial infarction, previous stroke and coronary reperfusion therapy are presented in n (%), not % (n).

All descriptions at Table 1 were reviewed, and all pointed information were appropriately modified.

Q.9. The number following ± or in ( ) should also be clarified.

This explanation was given at the first sentence of Statistical Analysis section: “Normally distributed data were presented as mean ± SD and skewed data as median and interquartile range (IQR).”