Authors’ Response to Reviewer 2 Comments

Thank you again for your thoughtful revision of our manuscript. Please find below our point-by-point responses to your comments. We hope that you will find the revised version of our manuscript suitable for publication in Nutrients.

Reviewer: The methods stating the sample size calculation is given in lines 103-111. The primary endpoint has then not been described yet, this should be done under statistical analysis.

You did not define your primary endpoint well; I presumed (and suggested) it was the prevalence of MetS at 3 months, and that you wanted to demonstrate a 10% difference in this endpoint. In your sample size calculation (lines 103-111) you inserted that you are looking for a 10% difference in all “following parameters … BP, TG, HDL-C, … and GLC”.

This seems incorrect, hope that you agree.

I have done a sample size calculation using the proportions of patients reaching the primary endpoint of MetS at 3 months, with patients in the intervention group having 10% less prevalent MetS than the patients in the control group, with the control group having an effect of 90% prevalence and the intervention group 80%. In that case, for a >80% power analysis, you would need 219 patients in each group to detect the difference of 10% with significance. What you have done is the sample size for a reduction of 90% to 57%: 33 patients in each group, or the sample size from 99% (no effect) to 72% in the intervention group: 33 patients in each group. You may choose which one you expected, I would choose the 90% to 60% prevalences. (But your study is underpowered to detect a 10% difference).

Please adapt these calculations in Methods and place sample size calculation under primary endpoint definition.

Authors: Thank you very much for your comment and the calculations performed. According to your suggestion, we have described the primary endpoint and modified the statements describing the sample size calculation. In the present study, the statistical testing was also based on testing the statistical difference between the intervention group and the control group (between-group difference), as well as on testing the within-group differences between the two time points (baseline and at 3 months) for the following parameters: BP, TG, HDL-C, LDL-C, TC, and GLC. The calculation was based on the assumption that the difference in the aforementioned parameters (not in the MetS prevalence) is 10%.

The following sentences now read as follows:

lines 99 – 112: "Sample size calculation was performed using a module Power analysis - Sample Size Calculation of the statistical software Statistica, version 6.1. (StatSoft Inc, Tulsa, OK, USA) [31]. The calculation was based on the impact of nutritional intervention on MetS and its components, for the two intervention arms. The MetS prevalence at 3 months was the primary endpoint for the sample size calculation and the calculation was based on the
expectation that the 3-month intervention program would result in the reduction of MetS prevalence from 100% to 57% in the intervention group (IG) and in the reduction of MetS prevalence from 100% to 90% in the control group (CG). Sample size calculation was also based on the fact that between-group and within-group difference of 10% in the following parameters is considered clinically significant: BP, TG, HDL-C, low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and GLC. With the estimated magnitude of significant difference of 10%, a standard deviation of 15%, and with a type I error rate (alpha) 0.05 and a power goal of 0.80, the threshold was 33 participants per group. The obtained value was increased by 15 - 20% due to the assumed drop-outs.

Reviewer: In lines 224-230 (newly inserted) you insert a post-hoc analysis calculation of statistical power. It seems somewhat superfluous, and the argument of being flawed is not a loss of statistical power when you do a per-protocol analysis, but an introduction of bias: who are these patients that drop out of the study, because they could have influenced your results. I would suggest to remove these lines.

Authors: Thank you very much for your comment. According to your suggestion, we have removed the sentences describing a post-hoc analysis calculation of statistical power (lines 231 – 237).

Reviewer: IN Methods, it has not been described how MetS is calculated (suggestion: after line 219). It is your primary endpoint, so either describe it or use a good reference.

Authors: Thank you very much for your comment. We have mentioned the criteria used for defining metabolic syndrome, along with the appropriate reference, under the Study participants and setting section. We agree that the aforementioned should be specified in detail and, therefore, we have inserted the following lines at the end of the Data collection and outcome measures section:

lines 220 – 226: “According to the Joint Interim Statement definition [30], participants were determined to have MetS if they had at least 3 of the following components: (1) elevated WC (cut-off points for Europids: ≥ 94 cm for men, ≥ 80 cm for women); (2) elevated TG (≥ 1.7 mmol/L) or drug treatment for elevated TG; (3) reduced HDL-C (< 1.0 mmol/L for men, < 1.3 mmol/L for women) or drug treatment for reduced HDL-C; (4) elevated BP (SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg) or antihypertensive drug treatment in a patient with a history of hypertension; (5) elevated fasting GLC (≥ 5.6 mmol/L) or drug treatment for elevated GLC.”

Reviewer: In Results, lines 303-308, you begin the sentence with “In addition ..”. However, these are your main results, so I would suggest to rephrase this: “The impact of the DASH diet on the prevalence of MetS and its features are shown in Table 3”.

Authors: Thank you very much for your comment. As suggested, we have rephrased the above-mentioned sentence (line 307).
Reviewer: In discussion, you use the words ‘moreover’ (line 358) and ‘furthermore’ (line 370), but these are words of argument, not of adding sentences together in time. So please begin line 358 with: “After 3 months” and line 370 with “The IG experienced..”

Authors: Thank you very much for your comment. According to your suggestions, the words “moreover” and “furthermore” have been removed. The following sentences now read as follows:

lines 353 – 354: “After 3 months, significant decrease in DBP was observed among the participants in the IG, when compared to the baseline values.”

lines 363 – 364: “The IG experienced a significant reduction in the MetS prevalence after 3 months.”