

Manuscript Number 16059/ Response to Reviewers

Response to Reviewer # 1 (03022543)

Thank you for reviewing the paper and for your good words.

Specific comments:

Figure 2: What is the P value for ALT, AST, GGT, ALP. Is there a statistically significant difference?

The reduction in ALT, AST, and GGT became statistical significant by the 3rd month of treatment (ANOVA for the 12 month period $p < 0.001$) and for ALP by the 6th month of treatment (ANOVA for the 12 month period $p = 0.01$). These are reported in the new Figure 2.

Response to Reviewer # 2 (02860798)

Thank you for reviewing the paper and for your good words.

Specific comments:

1. The reviewer thinks this finding is of particular interest, however it would strengthen the findings if the authors add more information about serum parameters not only showing transaminases.

Serum parameter changes during the 12 month of treatment are reported in Table 1 (see below).

2. Please add data about serum levels of inflammatory cytokines, free fatty acids, triglycerides and total cholesterol.

a. Serum levels of high sensitivity CRP at months 0, 6, and 12 are now reported in table 1.

Table 1: Changes in measured parameters during the study

| Parameter | Baseline | 1 st month | 3 rd month | 6 th month | 9 th month | 12 th month | P ANOVA |
|---------------------------|-------------|-----------------------|-----------------------|-----------------------|-----------------------|------------------------|---------|
| Age (years) | 40.5 ± 5.6 | - | - | - | - | - | - |
| Gender (male) | 16 | - | - | - | - | - | - |
| Cigarette smoking | 13 | 13 | 13 | 12 | 12 | 11 | NS |
| BMI Kg/m ² | 31.5 ± 1.1 | 31.3 ± 1.0 | 31.4 ± 1.0 | 31.6 ± 1.1 | 31.6 ± 1.2 | 31.5 ± 1.2 | NS |
| Waist circumference | 110.5 ± 6.1 | 110.4 ± 6.0 | 109.9 ± 6.1 | 110.6 ± 6.3 | 110.7 ± 6.2 | 110.4 ± 6.2 | NS |
| Total cholesterol (mg/dl) | 251 ± 22 | 226 ± 17 | 192 ± 16 | 185 ± 12 | 181 ± 8 | 179 ± 9 | <0.001 |
| Triglycerides (mg/dl) | 187 ± 19 | 161 ± 20 | 143 ± 26 | 123 ± 11 | 121 ± 22 | 117 ± 18 | <0.001 |
| HDL-cholesterol (mg/dl) | 38 ± 5 | 40 ± 5 | 42 ± 7 | 42 ± 4 | 43 ± 3 | 44 ± 5 | <0.001 |
| LDL-cholesterol (mg/dl) | 180 ± 23 | 152 ± 15 | 121 ± 17 | 118 ± 14 | 114 ± 9 | 110 ± 11 | <0.001 |
| hsCRP (mg/L) | 4.2 ± 1.3 | - | - | 2.7 ± 0.8 | - | 1.6 ± 0.5 | <0.001 |
| Serum creatinine (mg/dl) | 0.93 ± 0.2 | 0.92 ± 0.2 | 0.94 ± 0.2 | 0.92 ± 0.2 | 0.91 ± 0.2 | 0.90 ± 0.2 | NS |
| BUN (mg/dl) | 34 ± 8 | 34 ± 8 | 35 ± 8 | 34 ± 7 | 33 ± 6 | 31 ± 6 | NS |
| SUA (mg/dl) | 5.5 ± 1.1 | 5.4 ± 1.0 | 5.2 ± 0.9 | 5.0 ± 0.7 | 4.9 ± 0.8 | 4.8 ± 0.9 | =0.016 |
| Plasma glucose (mg/dl) | 102 ± 8 | 101 ± 8 | 96 ± 6 | 93 ± 7 | 89 ± 5 | 87 ± 5 | <0.001 |
| HbA _{1c} (%) | 5.3 ± 0.4 | - | 5.1 ± 0.4 | 5.0 ± 0.5 | 4.9 ± 0.3 | 4.8 ± 0.3 | <0.001 |
| Metabolic Syndrome | 20 | 20 | 18 | 9 | 0 | 0 | <0.001 |

Mean values ± SD. BMI = body mass index, HDL = high density lipoprotein, hsCRP = high sensitivity CRP, LDL = low density lipoprotein, BUN = blood urea nitrogen, SUA = serum uric acid, HbA_{1c} = glycosylated haemoglobin

b. Fatty acid levels were not measured

c. Baseline data and changes in triglyceride and total cholesterol levels are already reported in table 1 of the original manuscript.

3. Reduced inflammation after therapy should also be shown at histological level via F4/80 and/or CD11b staining. Reduced lipid accumulation should be confirmed via oil red o staining.

F4/80 and/or CD11b or oil red staining were not used because we considered the study to be of pilot nature. The reduced lipid accumulation is obvious even without oil red o staining. Now

that power calculations can be made and there is a description of the time frame of the changes it will be easier to plan (and budget) studies that will measure several additional variables including the ones listed by this referee.

Dear Prof. Yu

Thank you for your response.

We did not want to add the NASH scoring details because, as mentioned by the referee, the results are so obvious. However, since you requested that information we now include it in the text. This means that we added 222 more words. We also needed to add the reference used for NASH grading, in addition to the reference suggested by the referee.

I attach a clean copy as well as one with the new text (and references) highlighted.

I hope that this paper is now acceptable for publication.

Best regards

Dimitri

DP Mikhailidis BSc MSc MD FCPP FCP MFPH FRSPH FFPM FRCP FRCPath
Academic Head, Department of Clinical Biochemistry
Royal Free Hospital Campus,
University College London Medical School,
University College London (UCL),
Pond Street,
London NW3 2QG, UK