Author's response to reviews

Title: Effects of a 3-year dietary intervention on age-related changes in triglyceride and apolipoprotein A-V levels in patients with impaired fasting glucose or new-onset type 2 diabetes as a function of the APOA5 -1131T>C polymorphism: A 3-year intervention study

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Author's response to reviews: see over
Answers for Reviewers’ comments <Reviewer #1>

MS ID# : 6005154201117822 (1st revision)

Effects of a 3-year dietary intervention on age-related changes in triglyceride and apolipoprotein A-V levels in patients with impaired fasting glucose or new-onset type 2 diabetes as a function of the APOA5 -1131T>C polymorphism: randomized, open label, controlled study

Dear Reviewer #1,

We sincerely appreciate the time spent in reviewing this manuscript and your advice to improve it. Please, see below our answers to your queries and comments. We also marked the corrected and revised parts of the text in red. We hope that you find them satisfactory.

<Comments>

1. Is the question posed by the authors new and well defined?
   
   Reviewer #1: The questions defined by author are not entirely new, but are well defined.
   
   Answer) We sincerely appreciate the time spent in reviewing this manuscript.

2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?

   Reviewer #1: Yes, the methodology is well described and there is the assumption that it would be possible to repeat the experiment.
   
   Answer) We sincerely appreciate the time spent in reviewing this manuscript.

3. Are the discussion and conclusions well balanced and adequately supported by the data?

   Reviewer #1: the lack of people with CC allele
   
   Answer) In present study, among 203 Korean patients with IFG or new-onset type 2 diabetes, 91 were homozygous (TT) for the T allele, 98 were heterozygous for the C allele (TC), and 14 were homozygous (CC) for the C allele for the APOA5 -1131TC>C polymorphism. As a matter of fact, during the period from August 2006 to February 2008, approximately 17 subjects with CC allele were enrolled. However, following the exclusion criteria, subjects who intake any medications and does not match for the fasting glucose level were excluded. Therefore, we used only 14 patients
with homozygous for the C allele in IFG or new-onset type 2 diabetes for this study.

4. Do the title and abstract accurately convey what has been found?

Reviewer #1: Yes

Answer) We sincerely appreciate the time spent in reviewing this manuscript.
Dear Reviewer #2,

We sincerely appreciate the time spent in reviewing this manuscript and your advice to improve it. Please, see below our answers to your queries and comments. We also marked the corrected and revised parts of the text in red. We hope that you find them satisfactory.

<Comments>
Kim. Et al. presents an article entitled “Effects of a 3-year dietary intervention on age-related changes in triglyceride and apolipoprotein A-V levels in patients with impaired fasting glucose or new-onset type 2 diabetes as a function of the APOA5 -1131T>C polymorphism: randomized, open label, controlled study”

Even if, there are interesting results in this study that showed potential interaction between an intervention diet to reduce TG and -1131T>C genotypes in Korean subjects on triglycerides, major points have to be revised.

Major Compulsory Revisions

1/ Methods
This study is presented to be a randomized, open label, controlled study, in the title but in the study methods, it is neither a randomized study nor a controlled study. A group of 203 subjects was submitted to the same diet during 3 years, with no randomization between two diets or an intervention diet compared to a control diet. The title of the article is consequently inaccuracy.

Answer) In accordance with your advice, the authors decided to change the title more suitable for study design. To make it more clearly, we corrected the title to “Effects of a 3-year dietary intervention on age-related changes in triglyceride and apolipoprotein A-V levels in patients with impaired fasting glucose or new-onset type 2 diabetes as a function of the APOA5 -1131T>C
A control diet should have been very interesting to test the spontaneous TG and diabetes progression, and the genotype effect.

Answer) We applied the intervention program that replaced each subject’s refined rice intake with equal parts of legumes, barley, and other whole grains three times per day as high-carbohydrate sources and increased their vegetable intake to at least 6 units per day. In our previous study, we applied dietary intervention program that replaced 1/3 of refined rice intake with legumes three times per day as a carbohydrate source and increased vegetable intake to at least 6 units per day for sufficient dietary fiber intake. After 12-week dietary intervention, there were significant differences in levels of TG and HDL cholesterol ($P<0.001$, both) from baseline. In addition to this, subjects with TT allele had lower serum TG ($P=0.009$) and higher HDL cholesterol ($P=0.036$) than subjects with TC and CC allele [1]. Based on this study, our dietary intervention program was revised to increase consumption of protein and fiber for more dramatic effects of intervention, as replacing each subject’s refined rice intake with legumes, barley, and other whole grains instead of replacing only 1/3 of refined rice intake with legumes.

[Reference]

Moreover, the authors said that “a total of 203 subjects completed the study”. How many subjects were included at baseline and which percentage achieved the study?

Answer) In present study, among 203 Korean patients with IFG or new-onset type 2 diabetes, 91 were homozygous (TT) for the T allele, 98 were heterozygous for the C allele (TC), and 14 were homozygous (CC) for the C allele for the APOA5 -1131TC>C polymorphism. As a matter of fact, during the period from August 2006 to February 2008, total 244 subjects were enrolled (TT: 109, TC: 118, CC: 17). Approximately 83% of subjects achieved the study.

2/ the study involved subjects with impaired fasting glucose (IFG) or new onset type 2 diabetes. How many subjects were in each group and were there differences between genotypes subgroups? 
Answer) Among 192 subjects with IFG, 88 were homozygous (TT) for the T allele, 90 were
heterozygous for the C allele (TC), and 14 were homozygous (CC) for the C allele of the APOA5 -1131T>C polymorphism. In case of DM, total 11 were included; 3 with TT and 8 with TC. To increase statistical power, we pooled carriers of the less common allele (TC and CC). Likewise Table 2, the authors exhibited differences between genotypes in IFG.

<table>
<thead>
<tr>
<th></th>
<th>TT (n=88)</th>
<th>TC and CC (n=104)</th>
<th>P-value</th>
<th>P&lt;value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apolipoprotein A-V (ng/mL)</td>
<td>217.9±10.83</td>
<td>185.3±10.4</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Before&lt;sup&gt;2&lt;/sup&gt;</td>
<td>209.6±11.0</td>
<td>163.7±8.53*</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>After&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-7.38±11.2</td>
<td>-22.4±9.85</td>
<td>0.313</td>
<td>0.005</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>107.9±6.00</td>
<td>131.7±7.55*</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Before&lt;sup&gt;3&lt;/sup&gt;</td>
<td>102.4±6.86*</td>
<td>148.5±9.02</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>After&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-5.52±5.42</td>
<td>10.9±5.91</td>
<td>0.000</td>
<td>0.002</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>55.1±1.54</td>
<td>50.7±1.28</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>Before&lt;sup&gt;4&lt;/sup&gt;</td>
<td>33.7±1.47</td>
<td>47.9±1.47**</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>After&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-1.38±1.16</td>
<td>-2.85±1.17</td>
<td>0.378</td>
<td>0.068</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>108.4±0.67</td>
<td>107.0±0.58</td>
<td>0.548</td>
<td></td>
</tr>
<tr>
<td>Before&lt;sup&gt;5&lt;/sup&gt;</td>
<td>105.1±1.05***</td>
<td>107.0±0.92</td>
<td>0.081</td>
<td>0.046</td>
</tr>
<tr>
<td>After&lt;sup&gt;5&lt;/sup&gt;</td>
<td>-3.35±0.91</td>
<td>-5.06±0.87</td>
<td>0.047</td>
<td>0.048</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (mIU/mL)</td>
<td>9.17a±0.37</td>
<td>8.76a±0.37</td>
<td>0.291</td>
<td></td>
</tr>
<tr>
<td>Before&lt;sup&gt;6&lt;/sup&gt;</td>
<td>7.95a±0.37**</td>
<td>8.36a±0.39</td>
<td>0.485</td>
<td>0.237</td>
</tr>
<tr>
<td>After&lt;sup&gt;6&lt;/sup&gt;</td>
<td>-1.23±0.39</td>
<td>-0.39±0.37</td>
<td>0.124</td>
<td>0.045</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA-IR&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.47±0.11</td>
<td>2.34±0.10</td>
<td>0.267</td>
<td></td>
</tr>
<tr>
<td>Before&lt;sup&gt;7&lt;/sup&gt;</td>
<td>2.08±0.10***</td>
<td>2.22±0.11</td>
<td>0.398</td>
<td></td>
</tr>
<tr>
<td>After&lt;sup&gt;7&lt;/sup&gt;</td>
<td>-0.37±0.11</td>
<td>-0.12±0.10</td>
<td>0.045</td>
<td>0.048</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty acids (µEq/L)</td>
<td>535.4±22.9</td>
<td>515.1±20.1</td>
<td>0.554</td>
<td></td>
</tr>
<tr>
<td>Before&lt;sup&gt;8&lt;/sup&gt;</td>
<td>491.5±24.5*</td>
<td>495.9±22.7</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>After&lt;sup&gt;8&lt;/sup&gt;</td>
<td>-43.9±25.5</td>
<td>-19.2±26.0</td>
<td>0.501</td>
<td>0.656</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL particle size (nm)</td>
<td>23.7±10.0</td>
<td>23.7±0.08</td>
<td>0.670</td>
<td></td>
</tr>
<tr>
<td>Before&lt;sup&gt;9&lt;/sup&gt;</td>
<td>23.8±0.11</td>
<td>23.4±0.10**</td>
<td>0.009</td>
<td>0.005</td>
</tr>
<tr>
<td>After&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0.08±0.09</td>
<td>-0.26±0.09</td>
<td>0.012</td>
<td>0.048</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA (nmol/mL)</td>
<td>10.1±0.31</td>
<td>10.3±0.29</td>
<td>0.662</td>
<td></td>
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<tr>
<td>Before&lt;sup&gt;10&lt;/sup&gt;</td>
<td>10.8±0.40</td>
<td>11.8±0.35***</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>After&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0.62±0.38</td>
<td>1.50±0.33</td>
<td>0.079</td>
<td>0.048</td>
</tr>
<tr>
<td>Change</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ba-PWV (cm/sec)</td>
<td>2684.2±36.6</td>
<td>2648.3±35.1</td>
<td>0.465</td>
<td></td>
</tr>
<tr>
<td>Before&lt;sup&gt;11&lt;/sup&gt;</td>
<td>2705.0±40.1</td>
<td>2756.0±43.0**</td>
<td>0.464</td>
<td>0.282</td>
</tr>
<tr>
<td>After&lt;sup&gt;11&lt;/sup&gt;</td>
<td>20.8±29.7</td>
<td>107.6±24.1</td>
<td>0.023</td>
<td>0.028</td>
</tr>
<tr>
<td>Change</td>
<td></td>
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</tr>
</tbody>
</table>

The table shown in upper, differences between TT and C allele were almost similar as total subjects (Table 2). However, the authors could not make sure the p-values of DM, because of small sample size. A few subjects in DM group though; some markers had changed remarkably, however, the pattern of differences between genotype were similar as total in overall.
How many subjects with IFG became type 2 diabetic patients during the 3 year follow-up and were there differences between genotypes subgroups?

**Answer** The results of differences between genotype of subjects who progressed from IFG to DM were shown as bottom. Among 192 subjects in IFG, only 6 subjects with IFG became DM during the 3 year follow-up. To increase statistical power, we pooled carriers of the less common allele (TC and CC). However, the authors could not make sure the p-values, because of small sample size.

A pattern of differences between genotype had almost shown similar as Table 2, except fasting glucose, insulin, and HOMA-IR levels in both alleles were increased after 3 year follow-up.
At the end of the article (p16, l 349), the authors said that their results suggest that “the C allele contributes to the progression of diabetes”. The conclusion is not demonstrated in their results and should be modified.

**Answer** We found that the APOA5 -1131T>C polymorphism plays an important role in the metabolic response to a 3-year dietary intervention in patients with IFG or new-onset type 2 diabetes. Whole-grain ingestion prevented the age-related increase in triglyceride levels in patients with IFG or new-onset type 2 diabetes who carried the TT allele but not the C allele of the APOA5 -1131T>C polymorphism. As indicated in Table 2, fasting TG levels were reduced in TT allele carriers, while
it shows tendency of increasing in TC and CC allele carriers. Besides, high TG levels were considered a risk factor for diabetes, especially among subject who were obese. In this reason, we suggested that the C allele contributes to the progression of diabetes.

**Minor Essential Revisions**

1/ p 15, line 335.

There is a mistake in the discussion.

The C allele of -1131T>C polymorphism is not associated with a ribosomal translation efficiency. In literature, it is clearly showed that -1131T>C polymorphism is in strong linkage disequilibrium with 3 additional polymorphism: -3A>G, SNP2 and SNP1). It is -3A>G polymorphism, located in a Kozak sequence that has been possibly associated with a defect in translation efficiency but not confirmed up to date (Palmen et al.Biochim Biophys Acta 2008: 1782, 447-452).

*Answer* In accordance with your advice, we re-confirmed the reference that we mentioned, and found a mistake. As you pointed out, the -1131T>C change itself may not be associated with a ribosomal translation efficiency, but is acting as a marker for the -3A>G. The -3A>G change occurs in the ‘Kozak’ sequence in APOA5, and disruption of this six to eight base pair sequence that precedes the initiation methionine has been shown in other genes to severely disrupt ribosomal translation efficiency, and this would lead to lower levels of apo A-V from this mRNA [1]. Because the -1131T>C is in strong linkage disequilibrium with APOA5 -3A>G [1], apo A-V levels would be reduced in individuals with C allele. Therefore, we revised the manuscript as below.

“...This result suggests that if exercise is therapeutic only for abnormal lipid metabolism, then an innate human genome sequence for this effect may not exist. Previous research has suggested that the promoter variant -1131T>C is in strong linkage disequilibrium with APOA5 -3A>G, which occurs in the ‘Kozak’ sequence in APOA5, and disruption of this sequence has been shown in other genes to severely disrupt ribosomal translation efficiency, leading to lower levels of apo A-V from this mRNA. Therefore, the C allele may itself not be functional, but is acting as a marker for the -3A>G that may contribute to lower apo A-V levels [35]. More studies are needed to determine whether diet and exercise can improve apo A-V levels in individuals with the C allele.”

[Reference]

1. Martin S, Nicaud V, Humphries SE, Talmud PJ: *Contribution of APOA5 gene variants to plasma triglyceride determination and to the response to both fat and glucose tolerance*
2/ The association between apoAV level and TG levels in human is not clearly discussed, and compared to mice. Results are conflicting in literature with some positive and some negative correlations.

An interesting recent study should be discussed (Kim et al. J Clin Lipid 2013. 7, 94-101). This study showed an inverse correlation between apoAV and TG in -1131 TC and CC genotypes as in this study in 754 hypertriglyceridemic patients.

*Answer* In our previous research that you mentioned [1], we observed a negative correlation between apo A-V and TG in normotriglyceridemic controls and a positive correlation in hypertriglyceridemic cases, regardless of genotype. Instead, we observed lower apo A-V levels in CC allele carriers than in TT allele carriers, and association of the -1131T>C polymorphism with greater triglyceride levels, smaller LDL particle size, and lower HDL-cholesterol. We mentioned this reference in the Discussion section as follows.

“…Our data showed that the TT allele is associated with lower triglyceride levels compared with the C allele, which agrees with a study by Xu et al. that demonstrated higher levels of fasting triglycerides, total cholesterol, LDL, and HDL, as well as a 33% increased risk of metabolic syndrome, in 51,868 Chinese subjects with the C allele [8]. In our previous research, CC allele carriers showed lower apo A-V levels than TT allele carriers, and showed association with greater triglyceride levels, smaller LDL particle size, and lower HDL-cholesterol [23]. Similarly, Li et al. found a higher C allele frequency among individuals with high total cholesterol compared with individuals with normal total cholesterol levels in both ethnic populations (Hei Yi Zhuang and Han Chinese; P<0.05) [24]…”

[Reference]


**Could the authors give the correlation between apoAV and TG in each genotype groups?**

*Answer* Following your comment, we have made a table of relationship between in plasma apo A-V levels and TG in each genotype groups. These results were coinciding with previous study (J Lipid Res 2010; 51: 3281–3288).
3/ page 11 line 237
CC: 16.71±/-16.11.

**Is it 16.71 or -16.71 as suggested in figure 1?**

*Answer* The authors are sorry for making the reviewer confused. We corrected 16.71±16.11 to -16.71±16.11.

4/ the paragraph about relationship in plasma apoA-V and metabolic parameters is difficult to understand and redundant with table 3. It needs to be clarified.

*Answer* According to your advice, we condensed manuscript as follows.

“Correlations between the changes in apo A-V levels and metabolic parameters were determined after adjusting for age, sex, and changes in body weight ($r_1$), initial triglyceride levels ($r_2$), and initial apo A-V levels ($r_3$) (Table 3). Across all subjects, changes in apo A-V levels were negatively correlated with baseline apo A-V levels ($r_1=-0.543$, $r_2=-0.555$, all $P<0.001$). In TT allele carriers, changes in apo A-V levels were negatively correlated with baseline apo A-V levels ($r_1=-0.535$, $r_2=-0.552$, all $P<0.001$) and changes in triglyceride levels ($r_3=-0.299$, $p_3=0.006$). In C allele carriers, changes in apo A-V levels were negatively correlated with baseline apo A-V levels ($r_1=-0.613$, $r_2=-0.617$, all of $P<0.001$), but positively correlated with changes in triglyceride levels ($r_1=0.207$, $p_1=0.038$; $r_2=0.221$, $p_2=0.028$).”

5/ in the abstract ba-PWV and MDA should be explained.
Answer) Following your advice, we inserted explanation of abbreviated words in abstract as below.

“Our results showed that HDL, glucose, insulin, HOMA-IR index, free fatty acids, and apo A-V decreased, and brachial-ankle pulse wave velocity (ba-PWV) and malondialdehyde (MDA) increased at the 3-year follow-up visit compared with baseline…”
Answers for Reviewers’ comments <Reviewer #3>

MS ID# : 6005154201117822 (1st revision)

Effects of a 3-year dietary intervention on age-related changes in triglyceride and apolipoprotein A-V levels in patients with impaired fasting glucose or new-onset type 2 diabetes as a function of the \textit{APOA5} -1131T>C polymorphism: randomized, open label, controlled study

Dear Reviewer #3,

We sincerely appreciate the time spent in reviewing this manuscript and your advice to improve it. Please, see below our answers to your queries and comments. We also marked the corrected and revised parts of the text in red. We hope that you find them satisfactory.

<Comments>

The current manuscript authored by Minjoo Kim et al. described “Effects of a 3-year dietary intervention on age-related changes in triglyceride and apolipoprotein A-V levels in patients with impaired fasting glucose or new-onset type 2 diabetes as a function of the \textit{APOA5} -1131T>C polymorphism: randomized, open label, controlled study”.

Authors analyzed the effects of a 3-year dietary intervention on age-related changes in triglyceride and apolipoprotein (apo A-V) levels in patients with impaired fasting glucose (IFG) or new-onset type 2 diabetes as a function of the \textit{APOA5} -1131T>C polymorphism. Author also showed that the dietary intervention prevented an age-related increase in triglyceride levels in individuals with IFG or new-onset type 2 diabetes who possess the TT allele, but not the CT or CC allele, of the \textit{APOA5} -1131T>C polymorphism. The results seem sound and the methods used are in general valid. However, there’re several comments as below:

Major Compulsory Revisions:

1. Add the results about the independent predictor of triglyceride changes.

\textbf{Answer} According to your comment, the authors added the text of independent predictors of triglyceride changes at Results section in page 13.

“…To figure out the independent predictors of triglyceride changes, \textit{APOA5} -1131T>C genotype, age, baseline BMI, baseline apo A-V levels, baseline triglyceride levels, change in
triglyceride levels, change in HDL, baseline glucose, change in glucose, baseline HOMA-IR, and change in HOMA-IR were tested (data not shown). The APOA5 -1131T>C genotype emerged as an independent predictor of changes in triglyceride levels ($\beta=17.668\pm8.200$, $P=0.033$) along with baseline triglyceride levels ($\beta=-35.455\pm8.401$, $P<0.001$) and baseline apo A-V levels ($\beta=20.856\pm10.025$, $P=0.039$) across all subjects. Baseline triglyceride levels and changes in apo A-V levels also emerged as independent predictors of changes in triglyceride levels ($\beta=-36.641\pm11.743$, $P=0.003$ and $\beta=-0.179\pm0.068$, $P=0.010$, respectively) in both TT and C allele carriers ($\beta=-29.611\pm12.206$, $P=0.017$ and $\beta=0.182\pm0.078$, $P=0.001$, respectively). However, baseline apo A-V levels had shown as predictors of changes in triglyceride levels only in TT allele ($\beta=-31.465\pm13.668$, $P=0.024$).

2. The LDL-cholesterol was calculated by using the formula of Friedewald; therefore, describe the treatment of the subjects with serum TG >400 mg/dL.

Answer) LDL cholesterol was indirectly estimated in subjects with serum triacylglyceride concentrations <400 mg/mL using the Friedewald formula. In subjects with serum triacylglyceride concentrations $\geq$400 mg/dL, LDL was measured directly using an enzymatic method on a Hitachi 7150 Autoanalyzer. According to your advice, we inserted the direct method of LDL cholesterol measurement of the subjects with serum TG $\geq$400 mg/dL in method section as below.

“…LDL was indirectly estimated in subjects with serum triglyceride concentrations less than 400 mg/dL using the Friedewald formula: LDL = total cholesterol - [HDL + (triglycerides/5)]. In subjects with serum triacylglycerol concentrations more than 400 mg/mL, LDL was measured directly using an enzymatic method on a Hitachi 7150 Autoanalyzer (Hitachi Ltd, Tokyo, Japan)…”

3. Do authors have any data on the Coefficient of Variation (CV) for all the biochemical analysis used in the analyses? If available, please include the assay CV information in “Methods” section.

Answer) Following your comment, we supplemented inter and intra-assay precision (%CV) of biochemical parameters in Methods section. The inter and intra-assay precision of total cholesterol was 1.73% and 1.52%, HDL-cholesterol was 1.61% and 1.37%, triglyceride was 2.52% and 1.28%, glucose was 1.14% and 0.94%, insulin was 2.1% and 6.5%, hs-CRP was 2.08% and 2.46%, free fatty acid was 3.73% and 0.99%, apo A-V was 2.46% and 7.45%, MDA was 1.9% and 1.0%, and LDL particle size was 2.29% and 3.88%, respectively.

4. The authors performed multiple statistical testing. I strongly believe they needed multiple
testing correction rather than using 0.05 for significance.

**Answer** As reviewer knew that multiple testing corrections adjust p-values derived from multiple statistical tests to correct for occurrence of false positives. In microarray data analysis, false positives are genes that are found to be statistically different between conditions, but are not in reality. However, in present study, we did not testing several thousand of variables at the same time. Therefore, the authors feel that we did not need to do a multiple testing correction rather than using 0.05 for significance.

5. In Table 4. The change in HDL level is independent predictors of changes in apo A-V levels existed in all subject and also in both TT and C allele carriers. Please add the result and the possible mechanism in “Results section” and “Discussion section”, respectively.

**Answer** In accordance of your advice, we added result and possible mechanism in manuscript as follows in Results and Discussion section, respectively.

“…Baseline apo A-V levels and changes in triglyceride levels and changes in HDL-cholesterol levels also emerged as independent predictors of changes in apo A-V levels ($\beta = -112.767\pm19.550$, $P<0.001$; $\beta=0.404\pm0.191$, $P=0.038$; $\beta=3.280\pm1.007$, $P=0.002$, respectively) in both TT and C allele carriers ($\beta=-101.958\pm16.096$, $P<0.001$, $\beta=0.349\pm0.133$, $P=0.010$; $\beta=2.239\pm0.666$, $P=0.001$, respectively).”

“…Apo A-V would increase the binding of TG-rich lipoproteins to glycosaminoglycans present on the surface of endothelial cells, thus rendering these lipoproteins more accessible to LPL [36]. Therefore, decreased apo A-V levels may reduce LPL-mediated TG hydrolysis of chylomicrons and VLDL and delay the clearance of lipoprotein remnants by the liver [37]. After hydrolysis of TG-rich lipoproteins by LPL, surface components are detached to form native HDL of discoidal form [38]. Thus, the delayed TG hydrolysis, attributable to apo A-V deficiency, reduces the availability of surface components of triglyceride-rich lipoproteins, thereby leading to a decreased formation of HDL-cholesterol. In this way, apo A-V is associated with HDL formation, and this may result in significant contribution of HDL as independent predictors for changes in apo A-V levels…”

[Reference]


Minor Essential Revisions:

1. Dietary energy values and nutrient content from the 3-day food records were calculated using the Computer-Aided Nutritional analysis program (CAN pro 3.0, Korean Nutrition Society, Seoul, Korea), add the evidence or the literatures of reference.

*Answer* CAN pro 3.0 is commonly used nutrient analysis software in Korea, reflecting typical ingredients and amounts for Korean diet. Nutrient database for Can pro 3.0 is based on standard tables of food composition from Dietary Reference Intakes for Koreans (The Korean Nutrition Society, 7th edition), food composition table (Rural Development Administration, Korea, 6th edition), food composition table for Japanese (Japan women’s University for nutrition, 1997), and 17 other references [1].

[Reference]


2. In page 13, line 280. The “data not shown” must change to “Table 4”.

*Answer* According to your advice, we changed manuscript as below.

“Based on these results, we performed a multiple regression to determine independent predictors of apo A-V changes (Table 4)...”

3. In Table 4, why do the authors exclude the factors: baseline insulin, change in insulin, baseline ba-PWA, change in ba-PWA, baseline MDA, and change in MDA?

*Answer* A reason for exclusion of baseline and change in ba-PWV was not a variable which affects the changes of apo A-V levels independently. Reversely, apo A-V levels could affect the changes of ba-PWV, likewise triglyceride. However, when we added the factors of ba-PWV following reviewer’s comment (see below table), the results were similar as what we exhibited in Table 4.
The most widely used test for oxidative stress is measurement of MDA, a product of lipid peroxidation, by a thiobarbituric acid-reacting substances (TBARS) assay. However, the use of this assay to assess oxidative stress status is problematic because MDA is not a specific product of lipid peroxidation and the TBARS assay is not specific for MDA [1]. Thompson et al. [2] found that urinary MDA is a relatively insensitive marker for detecting differences in peroxidation. Also, an experiment in ischemic heart study, neither TBARS nor HPLC-authenticated MDA could be detected in heart perfused with 6 mM hydrogen peroxide [3]. Positive results with these assays were obtained only when the heart was perfused with 12 mM hydrogen peroxide, suggesting that TBARS and presumably MDA are generated in the heart only under extreme oxidative stress and not formed to detectable levels under mild or moderate oxidative stress. These observations underscore the need for more sensitive and selective methods for measuring lipid peroxidation. In addition, one of the prominent risk factors for increased lipid peroxidation is smoking. Because of the presence of free radicals in cigarette smoke [4], increases in MDA may occur [5-7]. Moreover, other environmental pollution may affect the levels of MDA. Therefore, MDA could easily affect by those of the conditions and following the suggestions of MDA which is insensitive and common peroxidation marker, the authors excluded MDA from multiple regression analysis. Besides, when MDA included, the results were fluctuating.

In case of baseline and change in insulin, these were already involved in HOMA-IR index;
\{\text{fasting insulin (µIU/mL) \times fasting glucose (mmol/L)}\}/22.5. Therefore, the authors decided to exclude insulin from regression analysis.

[References]


