Dear Professor Van de Werf:

Please consider our revised manuscript, “Dyslipidemia as a predictor of hypertension in middle-aged men”, for publication in the European Heart Journal.

We appreciate the interest that the editors and reviewers have taken in our manuscript and the constructive criticism they have given. We have addressed the major concerns of the reviewers. More specifically, we have shortened the introduction of the manuscript. As recommended by the statistical reviewer, we use only continuous variables for lipoproteins, apolipoproteins, and lipids in the analyses. This allows us to show the simple age-adjusted models for the association of baseline dyslipidaemia with new-onset hypertension in addition to the effect of adjustment for potential mediating and confounding variables related to lifestyle and the metabolic syndrome, as requested by the second reviewer. These changes have clearly improved our manuscript.

We have also included a point-by-point response to the reviewers in addition to making the changes described above in the manuscript. Changes to the text and footnotes of the tables in the manuscript are marked in bold.

Our main findings remain unchanged: dyslipidaemia characteristic of the metabolic syndrome predicts the development of hypertension, independently of features related to insulin resistance. We have measured apolipoproteins and the triglyceride content of HDL and LDL cholesterol in addition to traditional lipoprotein and triglyceride measurements. We have employed several complementary analytical approaches, including factor analysis. These findings are of potential importance for the prevention of hypertension and cardiovascular disease, and should be of interest to the readership of the European Heart Journal.

Thank you again for consideration of our revised manuscript.

Best regards,

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Comment. We thank the reviewers for the constructive criticism. The revised manuscript has a shorter, more focused introduction. We also present the analyses and findings in a clearer manner according to the suggestions of the reviewers.

Reviewer #1: Laaksonen and colleagues followed a cohort of 311 men and demonstrated that dyslipidemia is a risk factor for the development of hypertension.

Major comments
1. The external validity of this report is questionable. Indeed the authors recruited only men. Of the original cohort of 1038 men, they excluded all those with hypertension at baseline. The authors should compare the baseline characteristics of participants and non-participants. In addition, it would be interesting to know whether a cross-sectional analysis of the baseline data in all subjects confirmed the longitudinal results presented in the current manuscript.

Answer. We agree that a limitation of this study is the lack of women and other ethnic groups, as we state in the second-to-last paragraph of the discussion on p. 13). This was also a limitation in the study by Halperin et al. (ref. 5 in the manuscript), which was restricted to male physicians and in which only self-reported blood pressure was used. We do not consider the exclusion of men with hypertension a problem in a study examining the role of dyslipidaemia as a determinant of incident hypertension. We agree that exclusion of those with hypertension results in most of the men from this population-based study being excluded, but they are representative of middle-aged non-hypertensive men from Eastern Finland. Hypertension has a high prevalence in all Western countries. In Finland, high blood pressure is more prevalent than in many other western countries, but most middle-aged and older European men also have hypertension by current definitions.

As would be expected, men who were excluded because hypertension at baseline were slightly older, had a higher prevalence of cardiovascular disease, were heavier, and had a more pronounced dyslipidemia at baseline than those who did not have hypertension. In cross-sectional analyses of the association of the seven measures of lipid, lipoproteins and apolipoproteins shown in Table 3, the concentrations of serum triglycerides and apolipoprotein B and the triglyceride content of the LDL particles were associated with hypertension at baseline independently of CVD, diabetes, alcohol intake, smoking and adult socioeconomic status. When further adjustment was made for waist circumference, only serum triglyceride concentrations remained associated with hypertension at baseline. These cross-sectional results are perhaps not surprising, but only in prospective analyses can these relationships be analysed with respect to possible causality.
2. It is generally known that risk factors cluster within subjects. Moreover, the timeline of the age-related increases in lipid factors and blood pressure might be different. By and large, the authors have few direct arguments, even in a longitudinal analyses, to interpret dyslipidemia as a causal of factor in the pathogenesis in hypertension. The more likely explanation is that shared genetic and/or environmental factors explain the association between high blood pressure and dyslipidemia.

**Answer.** We think that endothelial dysfunction probably plays a role. Endothelial dysfunction is integral not only in the pathogenesis of atherosclerosis, thrombosis and insulin resistance, but also in hypertension (addressed in the Discussion on the bottom of p. 11 and top of p. 12, refs. 18, 19, 23). Findings from trials assessing the impact of statins and fibrates on cardiovascular disease outcomes have also found a small blood-pressure lowering effect from these medications (refs. 8-9 in the manuscript). We agree with the reviewer, however, that shared genetic and environmental factors and residual confounding may explain some of the results. We indirectly mentioned this in the second-to-last paragraph of the discussion in the original submission, but in line with the reviewer’s comments, we now state this more clearly in the second-to-last paragraph of the discussion on p. 13).

**Minor comments**
1. The Introduction can be substantially shortened.

**Answer.** In line with the recommendations of reviewers 1 and 2, we have now shortened the Introduction.

2. The authors might provide some additional information about the second-order factor analysis. Reading the third paragraph on page 9, did not clarify what exactly was meant. One has to go through the whole manuscript to understand the second-order factor analysis.

**Answer.** We added the following sentence to the paragraph on statistics (bottom of p. 7): “The promax rotation allows derivation of correlated factors, which can then be rotated in a second-order factor analysis.”
Reviewer #2: This was an interesting and clinically relevant paper on various lipid measures of dyslipidemia in relation to hypertension in a cohort of Finnish men. Overall, the authors have presented their data and discussion in a clear and straightforward manner. Some issues remain, however:

Comment. We thank the reviewer for the positive feedback. We agree that this paper will be of interest to the readership of the European Heart Journal.

(1) (Introduction pp 3-4) This section was a bit long; paragraph 2's description of the Halperin et al study could be boiled down to the later statements about the lack of data incorporating features of the metabolic syndrome. In paragraph 3, the authors should focus less on the underlying mechanisms among the lipids and lipoproteins and more on how they may be linked with either blood pressure or hypertension in observational studies.

Answer. Both reviewers are correct in noting that the Introduction could be shortened without loss of essential information. We have shortened the Introduction according to the suggestions of the reviewer.

(2) (Methods p 4) Minor point: "The recruitment." sentence is repeated and should be deleted.

Answer. Done.

(3) (Methods p 4) There is a sizable drop in the number of subjects after excluding baseline hypertension (n= 854 to 311), which raises the question of why the 311 men do not have hypertension - they are an initially healthier group. Men with baseline cardiovascular disease, however, should also be excluded out of concern for the impact of various treatments on lipids and BP. How do the results change with their exclusion? Finally, the authors do not indicate whether baseline lipid lowering treatment was considered in their analyses.

Answer. We agree that it would be desirable to exclude cardiovascular disease also, but the statistical power would be markedly decreased. However, there is no evidence of a statistical interaction between CVD at baseline and dyslipidemia with respect to the development of hypertension. For example, for the seven measures of dyslipidemia in Table 3, the P-value for the interaction term ranged from 0.14 to 0.83.

At baseline, none of the 311 men included in this study were using cholesterol lowering medication. We now state this in the manuscript (1st paragraph of the Methods, p. 4). This may seem surprising, but cholesterol-lowering medications
have become widely used in Finland only in the past 10 years or so, after the 4S trial was published in 1994. Baseline measurements were carried out in 1991 - 1994.

(4) (Methods pp 5-6) For baseline hypertension, was treatment current or current + past? For smoking status, past and never smokers should be distinguished.

**Answer.** Blood pressure treatment was current. We now state this in the definition of hypertension (p. 5, 1st paragraph). We now classify smoking as never, former and current smoking, now noted in the statistical methods section on the bottom of p. 6 and in the footnotes of Table 3. The breakdown is shown in Table 1.

(5) (Table 2) It would be informative to add diastolic blood pressure to this correlation matrix.

**Answer.** We added diastolic blood pressure to Table 2.

(6) (Table 3) It would be informative to provide the tertile cutpoints for each biomarker. While the description of the difference in RRs when adjusting for only age and SBP is helpful, it would be interesting to note the progression of RRs going from age-adjusted, then adding all traditional non-biomarker risk factors, then adding the highly correlated biomarkers, then adding SBP, which may reflect overadjustment since hypertension is the outcome of interest.

**Answer.** When using variables categorized into thirds, it would be difficult to display several models for the seven lipoprotein, apolipoprotein and lipid variables that we have analysed. In line with the request of the statistical reviewer, however, we now use continuous variables for the analyses presented in Table 3. As suggested by the reviewer, we also display the results for 4 different models in Table 3 and 5. Overall, models 2 and 3 give essentially the same results, but further adjustment for variables in model 4 attenuates the association of the variables slightly. We used the following models, as stated in the statistical analysis section (bottom of p. 6) and in the footnote under Table 3:

- **Model 1:** adjusted for age.
- **Model 2:** Adjusted for age and systolic blood pressure at baseline.
- **Model 3.** Adjusted for age, smoking (never-smoker, former smoker, and current smoker), alcohol intake (g/week), adult socioeconomic status, leisure-time physical activity, presence of cardiovascular disease, and presence of diabetes.
- **Model 4:** Adjusted for the variables in model 3 and waist girth, concentrations of insulin, glucose and C-reactive protein, maximal carotid intima media thickness and baseline systolic blood pressure.
(7) (Results) Do your results differ (effect modification or interaction) according to baseline SBP levels? While power may be limited, I suspect that the effect of lipids/lipoproteins/etc may be limited in a 7-year span to those with high-normal or prehypertensive BP levels.

**Answer.** Adjustment for baseline blood pressure has little effect on the results, as can now be seen in the revised Table 3. There was also no evidence of an interaction of baseline blood pressure with the lipoprotein, apolipoprotein and lipid variables with respect to incident hypertension. The reviewer is correct in that the statistical power is too limited for carrying out stratified analyses.

**Reviewer #3: Statistical Review**

>From a statistical point of view, I have the following main comments:

1. For the assessments of the univariate associations between lipids and hypertension, the authors have categorised these variables. While this certainly has advantages from a presentational point of view, from a statistical point of view, categorisation should be avoided whenever possible. Also, it makes it difficult to compare these results with the ones adjusted for confounding factors where lipids have been analysed using their original continuous scale. I therefore suggest the authors redo these analyses and assess the variable of interest as a continuous variable.

**Answer.** In accordance with the suggestions of the statistical reviewer, we now present all results using continuous variables for the lipoprotein, apolipoprotein and lipid measures. The overall results and main interpretation of the findings remain unchanged, although there are some minor effects on the statistical significance of some of the associations.
2. To assess the prognostic value of the variables of interest on the outcome, the authors have used model building procedures. From a statistical point of view, these procedures have several problems: firstly, due to the high amount of testing, there is an increased probability that chance predictors are included in the final model. Secondly, the strength of the association of true predictors with the outcome can vary considerably at each step depending on which (true and chance) predictors are included at that step. As a result, unless properly validated, the final model obtained by such procedures is known to be unreliable. Therefore, the authors should validate their model and its predictive accuracy. Alternatively, they could employ an epidemiological approach and correct their analyses for factors that are known to be important predictors of the outcome.

**Answer.** In line with the suggestion of the reviewer, we now show the results in Table 3 and 5 using 4 models, starting with simple age adjusted models and further adjusting for potential confounding or mediating variables. We originally analysed the data using similar models, but because of limitations in presentation of the data when using categorized lipid variables, we only showed the fully adjusted model in the original submission. When using continuous variables, it is possible to present several models showing the effect of successive adjustment for potential confounding or mediating variables, as suggested by reviewer 2. We think that use of continuous variables and presentation of the results using several models improves our manuscript.

All of the variables that have been employed in the models have been associated with hypertension, dyslipidemia and other features of the metabolic syndrome in prior studies. It is evident from the way the findings are now presented with four models that the addition of variables to the models for the most part has minimal effects on the associations presented. Also, using any combination of the individual covariates has little effect on the main findings. Some attenuation occurred in model 4 when adjusting for features related to insulin resistance and the metabolic syndrome. For lipoprotein and apolipoprotein variables having a weak association with incident hypertension with incident hypertension in models 1 – 3, the association was no longer significant in model 4.

In addition, I have the following minor and specific comments:

3. As the authors are aware, poor participation and/or attrition rates in a study can have major consequences on the results of a study and introduce selection bias. Therefore, more information should be provided to the reader in these two issues to enable the reader to judge whether such bias is an issue in this study.

**Answer.** We stated in the first paragraph of the Methods on p. 5 that “in all, 1038 participated in the baseline examinations, and 854 men (90% of those alive) participated in the 7-year follow-up.” This should allow the reader to judge whether bias is a major issue.
4. In the tables, present both counts and percentages for the categorical variables.

**Answer.** Done.

5. The authors should present summary statistics for the length of follow-up in this study sample.

**Answer.** The median length of follow-up was 6.99 y (interquartile range 6.74 – 7.25 y). The participants were examined in the same month as at baseline seven years later. This was not possible for many, but follow-up was within three months of seven years for 75% of the men. We now state this in the Methods (bottom of p. 4). Adjustment for the length of follow-up does not affect the results.

6. Throughout the manuscript, replace "multivariate" by "multivariable".

**Answer.** Done.

7. In the statistical methodology section, provide complete details on the multivariable regression analyses that were performed. Provide a list of variables that was assessed for inclusion into the model; rationale for the final model; description of how the linearity assumption was assessed and satisfied for all continuous variables; etc.

**Answer.** We now state in the statistical analysis section (top of p. 7) that “the linearity of the association of the lipid, lipoprotein and apolipoprotein variables was assessed by categorisation of the variables into thirds. The association of lipid variables appeared linear, except for triglyceride concentrations in which the middle and upper third of the concentrations were equally associated with a higher risk of hypertension. Therefore all lipid, lipoprotein and apolipoprotein variables were analysed using continuous variables.” We also briefly present the rationale for the models, which will be clear for the readers because of the well-described association of these variables with blood pressure, dyslipidaemia and features of the metabolic syndrome. We also assessed potential non-linear associations for other continuous variables used as covariates in the models, but we found none.