PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to the OEM but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open where it was re-reviewed and accepted.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Long-Term Exposure to Traffic-Related Air Pollution and Progression of Carotid Artery Atherosclerosis: A Prospective Cohort Study
AUTHORS	Gan, Wenqi; Allen, Ryan; Brauer, Michael; Davies, Hugh; Mancini, John; Lear, Scott

VERSION 1 - REVIEW

This manuscript received three reviews from the Occupation and Environmental Medicine but these referees have declined to make their reviews public.

VERSION 2 – REVIEW

REVIEWER	Nino Kuenzli, Deputy Director Swiss Tropical and Public Health Institute Basel
REVIEW RETURNED	19-Jan-2014

GENERAL COMMENTS	Although the revision has improved the paper, I am still a bit puzzled about the not yet covered issues. The fact that the study finds no association between smoking and atherosclerosis – now mentioned in the revised version – raises some red flags. How could one expect to see effects (across extremely small contrasts) of ambient air pollution if the known strong contrasts in exposure between smokers and non-smokers show no association? This needs to be addressed. Else, my conclusion is that the null findings are probably what one should expect given the extremely low levels and very small contrasts in exposure. It is a paper to proof the limitations of epidemiology if the fundamental methodological requirement of exposure contrasts cannot be fulfilled. I have nothing against "null findings" but in this setting it is of course a bit a provocation... I have reviewed an earlier version (submitted to OEM) of this same paper. Thus, I do not summarize those comments. The revised version has improved and addresses most of the comments well.

	However, the new information provided in the revision also raised new questions and I still have some issues already raised before: 1. The addition of stratified models is indeed very informative and relevant. It is not clear to me whether those interactions had also been tested in non-stratified data (dummy variables) and whether any of those interaction terms were significant. The discussion of the observed heterogeneity in the point estimates across some interesting subgroups (such as obesity) is a bit modest. Similarly, I think that the highly convincing evidence from animal studies on air pollution and atherosclerosis, with the interaction of obesity / fat diet, should appear in the discussion too. 2. In the discussion of the null findings, I still miss some very blunt and clear statements not just about the very low levels of air pollution in this study region, but in particular the extremely low contrasts between subjects. This is a very essential and major weakness of this study which really needs to be put in context. Do the authors expect to detect signals across such a limited range of very low levels of exposure? The IQR's are very small and the cumulated non-systematic measurement errors may be of similar size as the contrasts are. I checked the "no" box in the above question "is the study design appropriate to answer the research question" indeed only for that reason: is it really possible to investigate the air pollution / atherosclerosis hypothesis with some 600 people follow-ed up over 5 years in Vancouver? Or do we face the natural limits of observational science? 3. I would not really agree with the notion that the pollutant-based models are only a secondary analysis, thus, I would like to repeat that those are important. In fact proximity measures are very crude proxies and in future meta-analyses across published studies, those results are usually the least useful of all as "proximity" measures can never be generalized to "proximity" in other cities and studies (nor over time within the same region). Given the possible relevance of susceptibility factors, I felt that the interaction models (current Table 6 for proximity only) should also be shown for the pollutants, available for future comparisons across studies. It may be in the supplement. 4. How do the authors explain the very puzzling finding that atherosclerosis was not associated with BMI, smoking, physical activity and blood pressure? At least the first two – and in particular smoking – are expected to be associated with atherosclerosis, thus, null findings for those factors is really surprising. One should put this in context of the pollution findings too. 5. I referred to the validation r2 of the LUR models but those are still not made explicit in the discussion. The (non-systematic) errors also depend on the performance of those models. This may be very important in Vancouver in light of the very small contrasts across

	Vancouver. Minor point: The discussion hypothesizes that the trial interventions might have reduced progression in our study based on the five clinical trials. Those trials are all published and were indeed mostly null findings. However, there was some hard-to-explain interaction between the association of air pollution with progression and those (mostly null) interventions (i.e. the air pollution effects were basically seen in the intervention groups, not the placebo arms).

REVIEWER	Barbara Hoffmann IUF Leibniz Research Institute of Environmental Medicine, Düsseldorf, Germany
REVIEW RETURNED	27-Jan-2014

GENERAL COMMENTS	The discussion should include mentioning, that no established risk factor of atherosclerosis was associated with CIMT progression in this study. This clearly limits the explanatory power of this study, which should be acknowledged. Also, the methodological issue of repeated measurements in plaque free area, which was mentioned in my previous review, should be acknowledged in the discussion. Other than that, the authors appropriately answered to my earlier comments.

VERSION 2 – AUTHOR RESPONSE

Reviewer Name: Nino Kuenzli.
Deputy Director, Institution and Country, Swiss Tropical and Public Health Institute, Basel Switzerland.
Please state any competing interests or state 'None declared': None.

Dear Editor, although the revision has improved the paper, I am still a bit puzzled about the not yet covered issues. The fact that the study finds no association between smoking and atherosclerosis – now mentioned in the revised version – raises some red flags. How could one expect to see effects (across extremely small contrasts) of ambient air pollution if the known strong contrasts in exposure between smokers and non-smokers show no association? This needs to be addressed.
Reply: Based on our study sample, we did not find a significant association between cigarette smoking and carotid artery atherosclerosis. While we agree with the reviewer that smoking is generally associated with CIMT, our inability to observe such an association is consistent with the findings of a recent randomized controlled trial on the association between cigarette smoking and progression of CIMT (Johnson HM et al. PLoS One 2012). Nevertheless, it should be noted that in our study, there were only 35 current smokers (7% of participants). Such a small sample size was less likely to provide reliable results on the associations between cigarette smoking and carotid artery

atherosclerosis. We had provided this information in response to a previous reviewer's comment.

Else, my conclusion is that the null findings are probably what one should expect given the extremely low levels and very small contrasts in exposure. It is a paper to proof the limitations of epidemiology if the fundamental methodological requirement of exposure contrasts cannot be fulfilled. I have nothing against "null findings" but in this setting it is of course a bit a provocation...
Reply: We have discussed various possible explanations for the null results in the revised manuscript. While we agree that the lower levels and smaller contrasts of ambient air pollution in the study region might be the major reasons, we do not have a strong basis to suggest these as the only reasons for the null associations. In a large population-based cohort study conducted in metropolitan Vancouver, and using the same exposure assessment approach (Gan et al 2010, 2011, 2012), we found strong associations of coronary heart disease with traffic proximity and traffic-related fine particulate air pollution (black carbon). We thus performed the current study to further examine biological mechanisms for the observed associations. As discussed in the manuscript, in addition to the lower levels and smaller contrasts of ambient air pollution, the young age and absence of comorbidities of our study participants are also plausible explanations for the null associations. Further, as discussed in the manuscript, there are inconsistent findings regarding associations between air pollution and CIMT.

I have reviewed an earlier version (submitted to OEM) of this same paper. Thus, I do not summarize those comments. The revised version has improved and addresses most of the comments well. However, the new information provided in the revision also raised new questions and I still have some issues already raised before:
1. The addition of stratified models is indeed very informative and relevant. It is not clear to me whether those interactions had also been tested in non-stratified data (dummy variables) and whether any of those interaction terms were significant.
The discussion of the observed heterogeneity in the point estimates across some interesting subgroups (such as obesity) is a bit modest. Similarly, I think that the highly convincing evidence from animal studies on air pollution and atherosclerosis, with the interaction of obesity / fat diet, should appear in the discussion too.
Reply: In response to the suggestion, we performed the analyses to examine the significance of interaction terms; please see Table 6 in Page 26 for the revision. We also provided a paragraph to discuss the stratified analyses; please see Pages 19 for the revision.

2. In the discussion of the null findings, I still miss some very blunt and clear statements not just about the very low levels of air pollution in this study region, but in particular the extremely low contrasts between subjects. This is a very essential and major weakness of this study which really needs to be put in context. Do the authors expect to detect signals across such a limited range of very low levels of exposure? The IQR's are very small and the cumulated non-systematic measurement errors may be of similar size as the contrasts are. I checked the "no" box in the above question "is the study design appropriate to answer the research question" indeed only for that reason: is it really possible to investigate the air pollution / atherosclerosis hypothesis with some 600 people follow-ed up over 5 years in Vancouver? Or do we face the natural limits of observational science?
Reply: We agree with the reviewer. According to the comments, we revised the manuscript to

specify the lower levels and smaller contrasts of ambient air pollution in the study region. Please see Pages 3, 15, 18, 19 for the revisions.
As mentioned before, we performed the current study primarily because we found significant associations of coronary heart disease with residential proximity to road traffic and traffic-related fine particulate air pollution (black carbon) in a large population-based cohort study conducted in metropolitan Vancouver (Gan et al 2010, 2011, 2012). These previous findings indicate that adverse cardiovascular effects associated with exposure to ambient air pollution were present in the population of metropolitan Vancouver.
As discussed in the manuscript, we agree that our small sample size is a major limitation of the current study; however, this is only one of several plausible explanations for the observed null associations. Our analysis included 509 participants, while a recent study in Boston (Wilker EH 2013) reported a significant association between air pollution and CIMT in a sample of only 380 participants.

3. I would not really agree with the notion that the pollutant-based models are only a secondary analysis, thus, I would like to repeat that those are important. In fact proximity measures are very crude proxies and in future meta-analyses across published studies, those results are usually the least useful of all as “proximity” measures can never be generalized to “proximity” in other cities and studies (nor over time within the same region). Given the possible relevance of susceptibility factors, I felt that the interaction models (current Table 6 for proximity only) should also be shown for the pollutants, available for future comparisons across studies. It may be in the supplement.
Reply: We treated the analyses of traffic proximity as the main topic of this paper, because we previously found that living close to road traffic was strongly associated with the risk of CHD mortality, whereas changes in traffic proximity were associated with altered risk of coronary mortality (Gan et al 2010). Although we agree that traffic proximity is a crude surrogate for traffic-related air pollution, it is convenient, straightforward, and related to policy making. This method has been widely used in epidemiologic studies on health effects of traffic-related air pollution.
We would prefer not to provide stratified analyses for these four air pollutants for the following reasons: (1) As discussed in the manuscript, the small sample size is a major limitation of the current study, and the sample sizes for the subgroups are much smaller. Therefore the stratified analysis is less likely to provide reliable results. (2) Table 6 shows that for each subgroup, the effect estimates are quite different across different atherosclerotic markers. Because of very small sample sizes in these subgroups, it is difficult to tell that the heterogeneity is due to chance (small sample size) or due to real effects; the stratified analyses are difficult to interpret. Furthermore, the current study includes four traffic-related air pollutants, the data analysis and presentation for each of these pollutants will add a great deal of complexity to the paper. (3) Table 5 shows that there were no significant associations between these air pollutants and annual changes in carotid artery atherosclerosis; therefore, it is hard to support stratified analyses (please see Page 185 in Szklo and Nieto, Epidemiology: Beyond the Basics).

4. How do the authors explain the very puzzling finding that atherosclerosis was not associated with BMI, smoking, physical activity and blood pressure? At least the first two – and in particular smoking – are expected to be associated with atherosclerosis, thus, null findings for those factors is really surprising. One should put this in context of the pollution findings too.
Reply: In this study, we did not find significant associations of carotid artery atherosclerosis with

BMI, smoking, physical activity, or blood pressure. We think the young age and absence of comorbidities as well as small sample size of our study might be plausible reasons for the null associations. But we did observe significant associations of carotid artery atherosclerosis with age, sex, race, and LDL-C. As an observational study, it is difficult to clearly explain significant and non-significant associations; however, these results might provide some useful information. For example, we did not find a significant association between cigarette smoking and CIMT. This result is consistent with the null association between cigarette smoking and progression of CIMT in a randomized, double-blind, placebo-controlled clinical trial (Johnson HM et al. PLoS One 2012). These findings suggest that CIMT is not necessarily an ideal biomarker to reflect adverse cardiovascular effects associated with environmental exposure. We believe that future research is still needed to confirm the usability of CIMT in epidemiologic studies.

5. I referred to the validation r2 of the LUR models but those are still not made explicit in the discussion. The (non-systematic) errors also depend on the performance of those models. This may be very important in Vancouver in light of the very small contrasts across Vancouver.
Reply: Based on the comments, we provided the information on the performance of these models. Please see Pages 7-8 for the revision.

Minor point:
The discussion hypothesizes that the trial interventions might have reduced progression in our study based on the five clinical trials. Those trials are all published and were indeed mostly null findings. However, there was some hard-to-explain interaction between the association of air pollution with progression and those (mostly null) interventions (i.e. the air pollution effects were basically seen in the intervention groups, not the placebo arms).
Reply: Based on the comments, we removed the sentence. Please see Page 15 for the revision.

Reviewer Name Barbara Hoffmann
Institution and Country IUF Leibniz Research Institute of Environmental Medicine, Düsseldorf, Germany
Please state any competing interests or state ‘None declared’: None declared

The discussion should include mentioning, that no established risk factor of atherosclerosis was associated with CIMT progression in this study. This clearly limits the explanatory power of this study, which should be acknowledged.
Reply: We explicitly mentioned the null associations of CIMT with established cardiovascular risk factors, and briefly discussed the potential reasons in the revised manuscript. Please see Page 15 for the revision.

Also, the methodological issue of repeated measurements in plaque free area, which was mentioned in my previous review, should be acknowledged in the discussion. Other than that, the authors appropriately answered to my earlier comments.
Reply: We discussed the limitation in the manuscript. Please see Page 18 for the revision.