PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cannabis Exposure as an Interactive Cardiovascular Risk Factor and Accelerant of Organismal Ageing – A Longitudinal Study
AUTHORS	Reece, Albert; Norman, Amanda; Hulse, Gary

VERSION 1 - REVIEW

REVIEWER	Jouanjus, Emilie - UMR 1027: Equipe de Pharmacoépidémiologie, Université Toulouse III, UPS, Toulouse - Université Toulouse III, UPS, Toulouse - CEIP-Addictovigilance de Toulouse, Centre Hospitalier Universitaire, Toulouse, France
REVIEW RETURNED	13-May-2016

GENERAL COMMENTS	This manuscript is a research article dealing with the cardiovascular (CV) toxicity of smoked cannabis. The authors make the assumption that cannabis exposed patients may be aging more quickly than nonusers. This is a worthwhile subject, and a very topical issue, since the numerous debates surrounding the legalization of cannabis use, in therapeutics as well as for recreative reasons, in many countries worldwide. Moreover, WHO has just launched a report on the health effects of nonmedical cannabis use. The scientific community's awareness towards the CV risks of cannabis use has been increasing for several years; however, data clarifying what is exactly impacted by cannabis in the CV system are still lacking. Indeed, cannabis use is thought to be a triggering factor for CV diseases (such as stroke and acute coronary syndromes), but here the authors elucidate on this triggering role. The main result of this study is that cannabis accelerates vascular ageing: this is very informative, as it may (at least partially) explain why cardiovascular diseases that usually occur after a middle-to-long-term tobacco exposure are sometimes seen in very young cannabis users. This very informative result is indeed of considerable public health and regulatory importance, as the authors state in their conclusion, although I would say that before regulation, it is of considerable importance to health professionals. Here are my other comments and questions: - A specific paragraph presenting the health institution in which the study was conducted would be helpful in the methods section, since the fact that "patients were not selected" (page 7, line 8) is of varying importance depending on the number of patients treated there. - Monocentric design: Could the authors clarify on how what was observed in the cohort of patients managed in the primary care addiction treatment clinic of Brisbane, Australia, could be extrapolated to other cannabis users, in Australia or in other parts of the world? In other words: was the cohort representative? This is of crucial importance, as the authors introduce their manuscript

	presenting very general (ie, USA, world, but not Australia focused-) epidemiological data. - Exclusion/inclusion criteria: Amphetamine-exposed patients were excluded from the analysis, as were those exposed to alcohol, heroin or methadone. What about the cocaine-exposed patients? Considering the well-known pathophysiology of cocaine, I am surprised that this substance was included while other stimulants were not. This should be clarified in the Methods, and even discussed in the discussion section because it may have introduced a selection bias. - Introduction and Discussion/ References: Either the introduction or the discussion should refer to previous clinical studies specifically investigating the link between cannabis exposure and CV outcomes. Currently, these two sections present quite comprehensive information as pathophysiological factors are concerned, however, no or just a few pharmacoepidemiological data are given concerning what is already known about the health consequences of cannabis exposure. For example, such studies were conducted in France and are presented in the WHO Report. - Tables and figures: The figures are fantastic, although they are numerous. The manuscript would gain in clarity should the authors delete some of them. If I am not mistaken, figure legends lack of abbreviation definitions. To finish, it may sound as a mere detail, but I think the discussion should start with a sentence presenting the main result of the study. For example, this sentence could be: “The results demonstrate for the first time that patients exposed to cannabis demonstrate an advanced cardiovascular age in a longitudinal clinical series.” In its current form, it immediately puts the reader in a negative state of mind (and, once again, the size of the population treated in the health institution is lacking in the methods as here it would help to have an idea of the significance of “leaving” those 1,163 patients aside…).

REVIEWER	John Richards UC Davis Medical Center Sacramento, California, USA
REVIEW RETURNED	20-Jun-2016

GENERAL COMMENTS	Page 2, Line 20: I would mention here that (all?) participants were on concomitant buprenorphine. Page 4: There seem to be no limitations mentioned for this study under the Article Summary. For example, would the presence of buprenorphine be a potential confounder? (I realize you have addressed this much later and in a supplemental Table. Page 5, Line 13: add comma after (YLD). Page 6, Lines 26-30: Fragmented sentences. Page 7, Patient Selection: I would mention the subjects were also taking concomitant buprenorphine here. It’s unclear if the buprenorphine was discontinued at the 2 and 5 year marks or did some stay on it for a prolonged period. Page 7, RAPWT: Is there a reference or prior published study that describes or used this technique? At least one reference should be

	provided for this section. Page 7, Demographic: and Lab Data: Were the labs drawn at the initial visit or later? If multiple labs on same patient, were they averaged? If these are the labs included in Supplemental Table 6, I would mention that here. Page 9, Line 11: Delete “and so were excluded.” Page 9, Line 30: This is the first time in the paper you mention the patients are on concomitant buprenorphine. Although I agree that it is not a significant factor in accelerating VA or CA as you have explained it should be mentioned a s a possible confounder/limitation in the interpretation of results. Page 10, First paragraph: There are a few abbreviations that are not defined in the paper, example “loess” (LOcally WEighted Scatter-plot Smoother). Is there a reason the figures are not sequentially ordered – for example there are 2 SF6 figures (Pages 49 and 53). It makes it somewhat confusing. Page 10, Second paragraph: The full statistical results are included in the main text which may be overkill – these could simply be listed in the Figures/Tables with just the P values in the main text. Also there are a few abbreviations which are not defined “est., DF, AIC, BIC, Log. Lik.” Page 13: I would break this into at least 2 paragraphs. Page 17: References should be formatted to journal style. Page 21: Table 1 has a few cells with empty parentheses “()” – is this zero or just blank? Also periods are used instead of spaces in a few of the “Parameter” cells – “Heroin.Dose” – is there a reason for this? It is inconsistently used. Also your Tables and Figures should have a descriptive footnote summarizing the key findings and defining any abbreviations. This would be helpful to the reader as there is a large number of Figures and Tables. For example, this is the first time “THC” has made an appearance in the paper. Also the statistical test the P pertains to should be mentioned. Page 23, Table 2: See previous comments as they apply here – also what do the asterisks at the right pertain to? Page 25: As mentioned the Figures and Tables should have more descriptive captions. “Overal” is misspelled. Page 26: The inclusion of “Buprenorphine” here may not be necessary if you explain it in the Methods section – otherwise it is a bit confusing. Page 27: Figures should have more descriptive captions and all abbreviations defined “CA, RA, BMI.” What is the significance of the tilde “~” in this and some of the other Figures? It is not consistently used. Page 31: Reference should be formatted to journal style. Page 32: The upward arrow is used here – use superscript instead. See previous comments regarding abbreviations, statistics, footer,

	and asterisks. This also applies to the remainder of the Tables and Figures. Page 37: Again, there are many abbreviations here that need to be defined in a footnote. Especially so for the table that follows. Page 53: Is SF6 supposed to be SF10? There is no SF10. There is already a SF6 on Page 49. I would recommend sequential numbering for all Figures and Tables, even if they are included in the supplemental section.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1.

This reviewer has made many kind and thoughtful and appreciative remarks for which we are grateful.

I have added a paragraph relating to the specific health institution detailing in particular the volume of work we achieve as has been requested.

The issue of the generalizability of our patient cohort has been addressed in detail at the end of the discussion. This issue seems mainly related to cannabis exposure, and the level of cannabis to which our cohort was exposed is believed to be not atypical for many places. In making this remark it is important to note that the level of global cannabis exposure is likely rising, and also very variable.

Cocaine use in this country is very rare outside of certain suburbs in Sydney so it is not a confounder in our patients. Our patients were asked about this but universally denied it. This is now made clear at the commencement of the discussion.

Detailed references to the French epidemiological research and the recent WHO report on cannabis have now been included in the Introduction. I note that at the time of writing a pdf of this report has not been made generally available on the internet.

I accept the reviewer's observations in relation to the lack of explanation of the abbreviations. This shortcoming has now been amended throughout.

The sentence the reviewer has suggested with which to open the discussion has now been included.

Supplementary Figure 10 was mis-labelled as Supplementary Figure 6 and this has now been corrected.

Reviewer 2.

I have included a mention of the buprenorphine. Most but not all patients were on buprenorphine.

I have added a comma after YLD.

The error is sentence grammar at the end of the Introduction has been corrected.

As suggested I have added a reference for the RAPWT technique.

Blood for laboratory studies was drawn at study initiation and also as clinically indicated during normal medical care, and also updated annually or biannually for the normal clinical care of patients. These details have now been specified in the Methods sections. The comparative clinical laboratory data is only presented for the initial presentation for patients. This is now made explicit in the narrative description of the presentation of these results in the Results section. I have also clarified this issue in the Methods section.

Page 9 line 11 this phrase has been removed as suggested.

I have added buprenorphine as a possible limitation of the study in the penultimate paragraph in the discussion.

I have added details for the abbreviations mentioned. The word loess is explained as suggested at the foot of those supplementary figures where it appears. Explanations for DF, AIC, BIC and Log Lik have also been added to the Methods section.

Supplementary Figure 10 was mis-labelled as Supplementary Figure 6 and this has now been corrected. This corrections shows that the figures are now correctly in consecutive order.

As requested I have broken the first paragraph of the discussion section into two paragraphs.

The references have been formatted in accordance with the BMJ style.

The missing cells from Table 1 have been amended.

The abbreviation THC is explained in full at the bottom of this Table.
The periods have been removed as requested.

The asterisks have also been removed.

The captions of the figures and tables have been corrected.

The spelling of Overall has been corrected.

The headings of the Supplementary figures have been simplified. The narrations accompanying them are presented in the Results section.

The tildes have all be omitted with the simplificatoin of the titles of the supplementary Figures. (Its use is standard and widespread amongst statisticians). All the abbreviations have been explained – RA, CA, BMI etc.

References have been assigned to BMJ style.

Superscripts have now been used to replace the upwards arrow as requested.

I have corrected the abbreviations as suggested on page 37.

Supplementary Figure 10 was mis-labelled as Supplementary Figure 6 and this has now been corrected. This correction shows that the figures are now correctly in correct sequential consecutive order.