Author's response to reviews

Title: Cardiovascular risk estimated after 13 years of follow-up in a low-incidence Mediterranean region with high-prevalence of cardiovascular risk factors

Authors:

José M Huerta (jmhuerta.carm@gmail.com)
Maria J Tormo (mjose.tormo@carm.es)
Diana Gavrila (diana.gavrila@carm.es)
Carmen Navarro (carmen.navarro@carm.es)

Version: 2 Date: 12 August 2010

Author's response to reviews: see over
Dear Editor,

Plesae find below our response to the questions and suggestions arised by the reviewers of our manuscript #1057096355412666, entitled “Cardiovascular risk estimated after 13 years of follow-up in a low-incidence Mediterranean region with high-prevalence of cardiovascular risk factors”. We have addressed all the issues pointed out by the reviewers and include a point-by-point answer to every question, along with a revised manuscript version with changes highlighted in red.

We really hope the referees will find this revised version satisfactory.

With regards,
The authors.

Response to Reviewer #1.

This is a very well written manuscript that assesses the relationship between exposure to major cardiovascular risk factors and incidence of cerebrovascular and ischemic heart disease in a population-based cohort in Murcia, Spain. Study methods are correct and generally well described, with strict endpoint diagnostic criteria and classification procedures, and special attention given to statistical methods. Some limitations of the study (e.g. self-reported diabetes status, statistical power limitations) are adequately commented in the discussion section. Moreover, the study makes two important contributions to the literature in this field:

a) It shows, for the first time, that classical cardiovascular risk factors are associated not only with coronary disease, but with cerebrovascular disease too, in a Mediterranean region, such as Murcia, with a low morbimortality from cardiovascular diseases in the international context.

b) This study shows the feasibility of conducting large prospective epidemiologic studies using cross-sectional studies as baseline, and record-linkage techniques with administrative data for event detection in follow-up.

Mayor Compulsory Revisions

The lack of effect of obesity on vascular risk found in this study is surprising. Absence of statistical association may be due to:

1) categorization of BMI in obese/non-obese: a better approach would be to consider BMI in the continuous scale, in three categories as stated in the methods section (<25, 25-29.9, >=30), or in two categories (<25, >=25) which would probably improve statistical power.

We had analysed the effect of BMI with different approaches, but were unable to find an association neither with continuous nor in categorised variables. As the reviewer suggests, we now classify subjects according to their BMI as normal weight, overweight and obese. Null results are shown in Table 2.
2) Adjustment by intermediate variables, such as hypertension, triglycerides and diabetes. This gives an estimation of the independent effect of obesity, this is, the effect that is not mediated by these factors. In fact, data in table 2 show substantial reductions in RRs after multivariate adjustment, which probably would be more evident if considering other categories for BMI.

The reviewer is right that attenuation due to covariate adjustment may partly help to explain the lack of association with obesity. We have included a comment in the Discussion (page 13). However, BMI was not predictive of cardiovascular outcomes even in crude models.

It is suggested that authors include these considerations in the discussion section.

Minor Essential Revisions

Results in table 3 are not included in the text (results section)
We have commented the table (now Table 4) in Results. Thanks for pointing out this omission.

Discretionary Revisions (which the author can choose to ignore)
1. A brief but more detailed description of the recruitment methods would be desirable because the reference given in the text refers to an article in Spanish which many readers will not be able to understand.
   We have expanded the methodological section for a better explanation of recruitment methods.

2. It would be interesting to have a description of those subjects lost for follow-up, and also of those excluded because missing data. Can these exclusions bias the results? To what extent? In which manner?
   In response to the reviewers' suggestions we have included a supplementary table (Table S1) with the comparison between included and lost to follow-up participants. Also, major baseline differences between study participants and those who refused to provide a blood sample are summarised in the text. Unfortunately, since these participants lack information on the relevant variables for this study, we have decided not to include them in Table S1. A brief discussion about the possible consequences of selection bias has been included in the Discussion.

3. It would be interesting to know the number of missing values for every risk factor.
   Figure 1 shows the number of participants with missing information on biochemical variables, blood pressure levels of anthropometry (N=106). Table 1 now includes an 'unknown' category for those subjects with missing information on medication use, who cannot be defined as controlled/uncontrolled hypercholesterolemic or hypertensive. Because the number of participants with missing values is low (below 5%), we feel that information provided might be enough. For reviewer's information, among those who provided a blood sample (N=2420 participants), there were N=103 subjects with missing HDL-cholesterol concentration, N=14 with missing data on serum triglyceride concentration and N=1 participant for whom height was missing. Table 1 shows the number of participants with unknown medication status in regard to hypercholesterolemia, hypertension and diabetes.

4. Did results change substantially when using different cut-offs for risk factors (e.g., lower cut-off for BMI)?
   Results do not basically change when categorising the variables (cholesterol, triglycerides, BMI) according to different criteria, saved for those situations when the loss of statistical power may limit the ability to detect significant associations.
5. During the study period substantial reduction in risk factors prevalence and improvement in their medical control have occurred in Murcia. Can these changes have affected the study results?

As you comment, changes in risk factor profiles during the follow-up may differently influence the estimations of risk for each factor. Unfortunately, we have not repeated the measurement of exposures in the study participants at different moments in time, so it was not possible to evaluate the effect of this changes over time. A comment has been added in the Discussion (page 11).
Reviewer #2.

Manuscript: “Cardiovascular risk estimated after 13 years of follow-up in a low-incidence Mediterranean region with high-prevalence of cardiovascular risk factors”

Huerta and colleagues have performed a longitudinal retrospective-prospective population-based cohort study. Authors aims to estimate Hazard Ratio, Relative Risk and Proportional Atributable Risk for classical cardiovascular risk factors in Murcia, a region located in the Mediterranean context, with high prevalence of classical factors, but with still low burden of CVD. They found higher incidence than previously reported in similar epidemiological context for AMI. Most Ischemic Heart disease cases were mainly attributable to smoking, hypertension and hypercholesterolemia. They also report data on incidence of stroke.

Introduction:
Authors clearly identify the lack of prospective information in this geographical context.

Minor Essential Revisions
1. Page 3. 2nd par. Both, REGICOR [1] and SCORE [2] have been calibrations of Framingham and SCORE risk functions respectively, but only REGICOR have been validated [3] yet.
We have changed the sentence (page 3).

Methods

Major compulsory revisions
2. Please clearly state study design at the beginning of the methods section. It seems that there was no an scheduled prospective follow up, but a retrospective-prospective search of information. This design, retrospective-prospective cohort, has its own sources of bias, that should be briefly commented in the discussion section.
We thank the reviewer for his suggestion, but we have decided to maintain the definition of the study design as prospective (page4). Although not designed as such from the beggining, the study has a truly prospective design (meaning that all exposure variables and covariates were assessed before the onset of disease and could, therefore, not be influenced by the occurrence of the cardiovascular events). However, the retrospective search for outcome information has now been clarified in the text (page 6).

3. Statistical analysis.
a. Authors affirm “Kruskall-Wallis o # test were used to assess statistical differences”. Though people with enough statistical knowledge probably know that kruskall-wallis should be used instead of Analysis of Variance when requirements (homoscedasticity, gaussian distribution of residuals for each group to be compared, and similar sample size of the groups to be compared) are notsatisfied, readers should be given enough information about what for the method was used (homogeneity of proportions, means,...) and which variables were included in the comparison, although this was not the main statistical issue of the analysis.
See corrections in page 7.
b. There is no mention about population (World, European) used in age-standardized rates estimates mentioned in the 1st paragraph of the discussion section. This data was previously presented as not-shown information, and not many details were provided. In response to the reviewer interest, we are happy to present standardised rates of acute myocardial infarction and stroke for men and women, using weights either from the age distribution of the population of Murcia in the 2001 census or from the European Standard Population. This information is presented in the supplementary Table S2.

c. Hazard Ratio and Relative Risk are quite different things. In this sense there is no mention about how RR was obtained from HR, or if it was estimated as a ratio of cumulative incidences. The multivariate hazard ratios of acute myocardial infarction for each risk factor in Table 4 were used as an approximation to the adjusted relative risk of disease by levels of risk factor exposure, to better account for differences in covariates.

d. Authors write “Due to the limited number of …sex was used as a stratum in Cox model instead of presenting disaggregated results for men or women”. But sex is not an stratum, but a variable with two strata. Therefore all estimates are adjusted by age, but results are not “stratified” by sex, since there are not results by age and sex. Please also update table legends were “stratified by age and sex” appear.

Both sex and age (in broad categories) were introduced in the Cox models as stratification variables. This means that models are built to allow for different baseline hazard functions in men and women (and each age group), but present a final pooled estimate for both sexes combined. The STATA option strata() of the stcox command implements this analysis. If numbers were larger, we would have decided to present results separately by sex.

e. In the methods section, authors explain how heterogeneity by sex has been estimated (likelihood ratio). How did authors compare heterogeneity by sex between models for angina, stroke and myocardial infarction? Please explain. We have detailed the method for assessing heterogeneity in results due to sex in each model (page 8). No comparisons were made across models.

f. On the other hand prevalences of CV risk factors were obtained from an study where subjects recruited were aged 20 or more [4]. So that age and sex distribution, and probably other covariates distributions as well, is different from that in the sample for estimating Relative Risk. How this could affect PAR estimation? Did the authors use some kind of adjustment of their estimates? If not, please discuss (in the discussion section) how this could have an effect on your conclusions.

According to the reviewer suggestion, we have now standardised the prevalences obtained form the DINO Study in Murcia to the 2001 census population of the region (note that data for the DINO Study was gathered between 2001-2003) and truncated to the 20-70 years age group to better reflect the age range of the study participants (18-70 years) in whom hazard ratios were estimated. This way, the estimated prevalences and relative risks both refer to representative population from Murcia of approximately the same age range.

Minor Essential Revisions

4. Study sample: “all participants voluntarily agreed to take part in the study” Please confirm that they agreed to participate not only in cross-sectional [5], but
also to be contacted again for the follow-up. Participants agreed to participate and to allow the inclusion of their personal data in a database, registered as stipulated by law at the time of data recruitment. Access to hospital discharge registries, primary care databases and the National Death Index were granted by the competent Health Authorities. The study protocol was approved by the Ethics Committee of the Virgen de la Arrixaca Hospital, the main hospital in the Region of Murcia.

5. Variable definition.
   a. I am aware that at the time when cross-sectional study [5] was carried out, cut-off for considering high risk total cholesterol in primary prevention was 6.5 mmol/l (NCEP-ATPII). Anyway authors have lipids concentration at recruitment. Why did the authors use the 6.5 mmol/l cut-off for hypercholesterolemia definition instead of the currently accepted of 6.1 mmol/l since NCEP-ATPIII guidelines publication [6]. We have adopted this criterion, and modified the results accordingly.

   b. Since definition of hypertension is based on measurements at recruitment in 1992, please confirm that those subjects who were already diagnosed and treated at the time of recruitment, were considered to have hypertension regardless their blood pressure levels were bellow threshold (140/90) when recruited. Same for hypercholesterolemia and hypertriglyceridemia. Please also update table legends.

   c. Variables Educational level, and physical activity are not defined. These two variables are probably well describe in the article with results of the cross-sectional study [5]. But since this article is in Spanish, it would be convenient to include a brief description of them in this section. This is especially important for physical activity, since in Table 1 of the results section it is not clear whether there is one (with several levels) or two (moderate and intense) with several categories.

   We have included covariate definitions in the methods section, as requested (page 5). In our previous approach, we defined hypercholesterolemia and hypertension strictly according to blood levels, after we checked there were no differences in risk between healthy and diagnosed patients when cholesterol and blood pressure values were below the clinical risk threshold. Now, we have decided to include a separate table showing this information so that all results are provided to the reader: a) conditions defined by analytical results (Table 2), b) conditions defined separately by diagnosed/undiagnosed controlled/uncontrolled status (Table 3), and c) conditions defined either by the analytical results, a previous diagnosis or use of specific medication (Table 4), as stated in the ‘Definition of risk factors’ paragraph in page 6.

6. Statistical analysis

   d. Authors stated that “no significant violations of the proportionality assumption were detected”. This means that there is no significant changes in covariates during follow-up, and this is essential for using Cox model instead time-dependent Cox models, where we can introduce covariate changes with time. Since we are working with cardiovascular risk factors, it is quite possible and probable that prevalence of covariates change, and what is more important, that the magnitude of changes is probably different between covariates. Could you please explain how did you evaluate proportionality assumption, and what did you actually find?

   Violations of the proportionality assumption were tested on the basis of the slope of Schoenfeld residuals linearly modelled as a function of time (under the null hypothesis of proportional hazards, the curve is expected to have a zero slope). Partial and global tests for each outcome model showed
no statistically significant violations of the assumption (please, see text in page 7). Since no repeated measurements were carried out, covariates were entered in the models as fixed instead of dependent on time.

e. Authors clearly explain how they have obtained PAR. Although is clear in ref n.5, please, include in the text that prevalence for traditional risk factors come from the same region, Murcia.

See changes in the text (page 8).

Results
Well explained, maybe too short. But this depends on the format of the article (brief?). Anyway most results are well presented in tables.

We have briefly expanded the Results section.

Major compulsory revisions
7. Age-standardized rates are mentioned at the beginning of the discussion section, but not in the results sections.

Age-standardised rates are presented as supplementary data in Table S2.

Minor Essential Revisions
Discussion
8. Main finding is clearly stated, but as I write above, there is no mention to these important estimates (age-standardized rates) at the results section.

Please, see above.

9. To make it more readable, it would be convenient to reorder the paragraphs putting together those about cerebrovascular disease, and those about ischemic heart disease. For example, paragraph n. 4 “virtually no data…” should finished at “…precludes direct comparisons with the present study.”, and moved after “…CV risk assessed for diabetes might be underestimated and should be considered with caution.” Since this paragraph is about AMI.

The paragraph has been reordered as suggested. Thanks.

Major compulsory revisions
Since PAR depends on the prevalence of the condition, using cut-off 6.5 mmol/l for hypercholesterolemia could have artificially reduced PAR for this risk factor. Please discuss briefly.

We have modified our criterion for better comparison with current and future PAR estimates in other settings.

10. About the effect of selection bias on the RR estimates, I believe that over or underestimation it is not due to the fact that “fewer hypercholesterolems and more ever-smolkers among participants lost to follow-up…”. We cannot predict under or over-estimation of RR since we cannot ascertain the incidence ratio among those who were not eventually recruited. It doesn’t matter whether hypercholesterolemia is more or less prevalent among those lost to follow-up. The important issue is to know whether the relationship (ratio) between incidence of the event (AMI, angina, stroke) within hypercholesterolemic group and incidence among non hypercholesterolemic group keeps the same among those who were not selected. Since we don’t have this information (outcome) we are unable to predict the effect of this selection bias.

A comment has been added to the Discussion (page 13).
11. Please also include a brief discussion about whether and how information bias, mainly due to retrospective-prospective nature of the study, could have distorted association.
Briefly mentioned in the Discussion (page 14).

Minor Essential Revisions
12. Given that high proportion of subjects with missing values (775/3089, 25%) plus those lost at follow-up (291/3089, 10%), it should be advisable to include more information about baseline differences between those subjects that were eventually included and those who were not, in order to evaluate potential selection bias.
We have added these data in Table S1. Please, also see response to Reviewer #1.

13. Authors affirm: “Virtually no data exist in Spain on the prospective association between CVRF and incidence of ischemic or cerebrovascular events”. Unfortunately there is no mention to age-standardized rates for stroke, neither at the beginning of the discussion, nor in the results section. Please provide.
We are happy to provided these data in Table S2.

Tables and figures
Table 2.
14. Too large. Many RR and 95%CI occupy several lines.
Table 2 has been shortened and univariate analyses have been suppressed to make it more readable.

References
15. Reference n 18. Title should be in bold.
16. Reference n 19 “Edited by” is repeated.
We have corrected these mistakes. Thanks for spotting.