Author’s response to reviews

**Title:** Homocysteine and education but not lipoprotein (a) predict estimated 10-Year risk of cardiovascular disease in blood donors. A community based cross-sectional study

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Reviewer 1

Vadini and collaborators evaluated the correlation between homocysteine, lipoprotein A, education level and a 10-year risk of CVDs in blood donors. This is an interesting study in a unique population. I have no significant remarks about the methodology. However, it is not clear why patients aged between 18 and 69 years were enrolled in this study if CRS is not applicable for subjects under the age of 35 years. In the opinion of the reviewer the most interesting conclusion of the study is an unexpectedly high CV risk in blood donors. This topic should be more elaborated in the discussion section, more studies involving blood donors should be discussed. The relatively high CV risk in this population could also be reflected in the title of the paper. On the other hand, it seems not surprising that higher education was associated with lower CV risk, as it is usually associated with "a healthier lifestyle". Finally, speculations on the cognitive reserve volume and its possible mechanism responsible for the reserve, should be shortened as this is not the topic of the study.
R: We thank the reviewer for his/her appreciation of the manuscript and for important comments. We enrolled patients between 18 and 69 years since this study is part of a project aimed at evaluating traditional and non-traditional cardiovascular risk factors, including homocysteinemia and lipoprotein (a). For greater clarity we have added this endpoint as the primary objective of the study (page 4, lines 78-80). Secondary objective was to estimate 10-yr cardiovascular risk. Therefore, all active donors who had reached the age of eighteen (minimum age for eligibility for blood donation) were consecutively enrolled, applying the 10-year CV risk estimate only to donors with an age equal or greater than 35 years.

Moreover, as correctly suggested by the reviewer, we have expanded the discussion about the higher-than-expected prevalence of cardiovascular risk in blood donors (page 10, lines 221-222 and page 11, lines 229-239). This observation per se is not completely novel, since an Italian study carried out on over 11,000 blood donors has shown comparable results in terms of estimated risk at 10 years (ref. 22). This consistency with previous studies improves the robustness of our findings. At variance, evaluation of CV risk biomarkers in healthy donors is an original finding, which we intended to emphasize in the title.

Finally, we have now shortened the discussion concerning cognitive reserve, as suggested.

Reviewer 2

Reviewer 2: In their cross-sectional study Vadini et al. investigated if homocysteine, education and lipoprotein (a) predict an estimated 10-Year risk of cardiovascular disease in blood donors. The aim of the study is interesting. However, I have the following comments:

1. It seems that blood pressure was measured only in one arm which can lead to underestimation of the results. It remains unclear on how many readings blood pressure was averaged or whether additional readings obtained from the sphygmomanometer have been taken in this analysis. This needs to be addressed in the manuscript.

R: We thank the reviewer for the important comments and suggestions.

We followed the latest European Society of Cardiology guidelines at the time of the study (2013 ESH/ESC Guidelines for the management of arterial hypertension; Eur Heart J 2013;34:2159–2219). At least two blood pressure measurements were taken, in a sitting position, 1-2 minutes apart, and additional measurements if the first two were quite different. We have considered the average value of the different measurements. Unfortunately, blood pressure was taken only in one arm. This has now been specified in the methods section (page 6, lines 116-119).

2. The paragraph regarding the cognition should be shortened or removed from discussion as cognitive performance was not tested in the present study.
R: We have removed a large part of the discussion on cognitive reserve.

3. Authors should add to the manuscript the strengths and limitations of the present study.

R: The strengths and limitations of the study have been included in the discussion section (page 12, lines 276-278, page 13, lines 279-284).

Minor Comments: Please check carefully spelling; in the Abstract, Conclusion section: the sentence starts with small letter (i.e. our study..).

R: We thank the reviewer for checking the spelling and highlighting typos, that we have now amended.