Dear Professor Fang-Fang Ji and reviewers.

We want to thank reviewers and the editorial board for all the comments about the article "Subclinical carotid atherosclerosis predicts all-cause mortality and cardiovascular events in obese patients with negative exercise echocardiography". We truly believe that these modifications have improved the quality of our manuscript.

Main Changes introduced:

The methods and results sections have been modified.

The discussion section has been also modified to introduce comments related to reviews' suggestions. Some changes in phrases in our comments to reviewers in the final text could be found due to cross check revision of the text

Author´s responses to reviewers' comments:

RV: Reviewers' comments.

AA: Authors' Answer.

AUTHORS´ REBUTTAL NOTE – Reviewer´s code: **02465908**

RV_02465908 comment 1: In the methods sections, authors reported precisely reasons for exclusion of patients from this study in the text as well as in figure 1, however they summarized in a few words parameters listed in table 1. In my opinion how and when clinical characteristics were collected and calculated should be explained, especially considering that glomerular filtration rate and metabolic equivalents were found to be independently associated with adverse events.

AA_comment 1:

Clinical characteristics were recorded at the time of first medical visit and treatment data were obtained at the first visit after EE performance. In the methods section, study population subsection we have added the next paragraph: "***Demographic and clinical characteristics as well as CAD pre-test probability (PTP) were collected from medical records at the time of first medical visit when EE was requested. Baseline echocardiography, carotid ultrasonography and stress testing data were collected from digitally stored images and medical records at the time of EE performance. CAD pre-test probability (PTP) and Systematic COronary Risk Evaluation (SCORE) were assessed according to current European Society of Cardiology guidelines. Treatment data were collected from medical records obtained at the first visit after EE performance. Of the 226 patients 172 (76.1%) were evaluated the same day after EE performance, for the 54 patients not evaluated in the same day the median time between EE and first medical was 13.5 [interquartile range 47.3] days.***

RV_02465908 comment 2: Was diabetes included in the multivariate analysis model? Moreover oral antidiabetic drugs and insulin treatment should had been prescribed before exercise stress echocardiography (as stated in table 1).

AA_comment 2:

Multivariate analysis was performed using Cox's proportional hazards models backward stepwise selection analysis with an entry set at 0.2 significance level and a retention set of 0.1. A p value of <0.05 was considered statistically significant. Diabetes mellitus was not associated with adverse events in Cox's proportional hazards univariate analysis (Hazard ratio 2.52, 95% confidence interval 0.60 – 3.38, p=0.427). However, we have

performed another multivariate analysis including diabetes mellitus without finding any differences to the previous reported in the article.

RV_02465908 comment 3: It has been reported that ultrasonographic evaluation of carotid arteries damage is related to coronary atherosclerotic damage also in dialysis patients (Int J Artif Organs 2007; 30 (444): 315-320). Moreover relationship between adverse events and kidney function should include evaluation of proteinuria (the latter could be related to presence of diabetes). Diabetic patients with proteinuria have high risk for cardiovascular events. Finally atrial dilatation was not included in the analysis. I wonder if all these points could be included in the discussion section as limitations of the study.

AA_comment 3:

We have rebuilt discussion section referred to chronic kidney disease following reviewers' advices. In page 11 line 24 we have change the following text: "***It was not surprising to find glomerular filtration rate and mitral valve regurgitation as AE predictors. Several articles have found a significant relationship between CP and/or CIMT and CAD presence and extension in dialysis or end stage renal disease patients[34, 35]; moreover renal disease has been associated with worse prognosis after an acute coronary syndrome[36]. Focusing in obese patients with angiographic CAD, chronic kidney disease, defined as glomerular filtration rate <60 mL/min/1.73 m2, was a strong predictor of cardiac events (hazard ratio 1.63, 95% confidence interval 1.05 - 2.53) and overall mortality (hazard ratio 2.17, 95%, confidence interval 1.54 - 3.07) in Asiatic subjects with BMI >25 kg/m^2[37].".***

Related to proteinuria, it is retrospective cohort study and only 123 patients (54.4%) had this data available. This is the main reason because proteinuria was not included in the statistical analysis. This fact has been included in study limitations (page 12 line 18): "***The main limitation of our study is that it is a retrospective and a single-centre study. For that reason, circulating or urinary biomarkers that may be useful to guide therapy in specific circumstances (e.g. albuminuria in hypertension or DM may predict kidney dysfunction and warrant renoprotective interventions) were not analysed, but this strategy is in consonance with 2016 European Guidelines on cardiovascular disease prevention in clinical practice where routine assessment of circulating or urinary biomarkers is not recommended for refinement of cardiovascular risk stratification (class III B recommendation)[1].".***

RV__02465908 comment 4: Minor remarks Abbreviations should be explained in all tables Dependent variable should be reported in table 3 and table 4.

AA_comment 4:

Abbreviations have been explained in the tables.

In table 3 and 4 dependent variable is "adverse events" and it is specified in the title.

AUTHORS´ REBUTTAL NOTE – Reviewer´s code: **03702209**

RV_03702209 comment 1: Some of The patients included in the study may have healthy metabolic obesity i.e. have a lifestyle that involves exercise activities. The level of exercise in the cohort is not recorded. The only clue of "fittness" is a negative EE.

AA_comment 1:

Cardiorespiratory fitness in these patients is expressed in metabolic equivalents (MET) performed at the EE. A MET is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 ml O^2 per kg body weight x min. The MET concept represents a simple, practical, and easily understood procedure for expressing the energy cost of physical activities as a multiple of the resting metabolic rate and it is widely used as a marker of cardiorespiratory fitness status. According to 2007 American Society of Echocardiography Recommendations for Performance, Interpretation, and Application of Stress Echocardiography a normal exercise echocardiogram result with good exercise capacity (7 METs men and 5 METs women) is associated with very low risk (<1% per year) for cardiac events. In this sense the mean METs of our sample was 8.5 (2.9) METs and METs were associated with lower rate for adverse events. We believe that cardiorespiratory fitness is well defined in our population.

RV_03702209 comment 2: In page 2 (abstract) and page 7 define "metabolic equivalents-METS 3.

AA_comment 2:

We refer the reviewer to AA_comment 1.

RV_03702209 comment 3: In page 3 line 27 replace this by these.

AA_comment 3:

we have replaced this by this as reviewer suggested.

RV_03702209 comment 4: In page 6 line 14 clarify the phrase "expert cardiologists blinded to the angiography results". Did all subjects undergow invasive angiorgaphy???

AA_comment 4:

It was a mistake, thanks for highlighting this, the correct sentence, that has been added to the paper, is ***"Both EE and carotid ultrasonography stored images were analysed by two imaging-expert cardiologists blinded to the adverse events. In case of disagreement, a third expert was consulted.".***

RV_03702209 comment 5: in page 8 line 29 clarify the phrase "similar findings were obtained in ischaemic patients". How was ischaemia defined in the patients of the Manuscript Review study 6. in page

AA_comment 5:

It refers to the studies mentioned after the point. We have added the references to avoid misunderstanding. In page 9 line 12 we have rebuilt the following sentence ***"Our study shows that CP increased by 2.26 the probability of an AE in obese patients with CAD suspicion and negative EE; similar findings were obtained in other studies performed in ischaemic patients[24-30]."***

RV_03702209 comment 6: in 12 line 1 define what PTP stands for i.e. pre-test probability

AA_comment 6:

Pre-test probability of CAD is based in the article "A clinical prediction rule for the diagnosis of coronary artery disease: validation, updating, and extension" (Eur Heart J 2011;32:1316–1330) that is followed by European guidelines on stable coronary artery disease and it reflects the likelihood of having significant CAD according to age and symptoms. Coronary artery disease pre-test probability is defined in methods section, page 5 line 19: ***"Demographic and clinical characteristics as well as CAD pre-test probability (PTP) were collected from medical records at the time of first medical visit when EE was requested.".***

AUTHORS´ REBUTTAL NOTE – Reviewer´s code: **03846820**

RV_ 03846820 comment 1.a.: Please, optimize your general concept with the proper Introduction and definitions: a) There must be clear understanding about the link between subclinical atherosclerosis and obesity. Please, elaborate it according to the previously published papers such as 10.1016/j.atherosclerosis.2017.03.035, 10.1161/JAHA.114.001540.

AA_comment 1.a.:

We have rebuilt the introduction according to reviewer advice. The introduction is divided in 3 sections:

1- Obesity as a health problem and its association with cardiovascular disease.

2- Carotid disease as marker of overall mortality and cardiovascular events.

3- Studies relating carotid disease and adverse events in obese patients and lack of evidence.

RV_ 03846820 comment 1.b.: Do you think it could be better to use a definition of "subclinical carotid atherosclerosis" in the title and throughout the manuscript.

AA_comment 1.b.:

We have changed the title according to reviewer's suggestions and it is defined in the methods section carotid ultrasonography subsection: **"Subclinical atherosclerosis was defined as a binary variable as CP presence/absence".**

RV_ 03846820 comment 1.c.: page 3 - you write "As we previously described" without any reference! I would remind that the only novelty of your manuscript is that dimension of the CV events in patients with obesity and subclinical carotid atherosclerosis with healthy stress echo. Please, harmonize it.

AA_comment 1.c.:

This sentence refers to "**Several epidemiological studies have demonstrated an independent association of carotid disease, defined as carotid plaque (CP) or carotid intima media thickness (CIMT), with overall mortality and cardiovascular events[12-15].".** We have added the references again after "**previously described"** to avoid misunderstanding.

RV_ 03846820 comment 1.d.: IMT - this is one of the most challenging options in the article. Please, be careful and accurate with your judgments and comments. I would suggest you to harmonize your Introduction and Discussion in accordance with the modern-day understanding of its clinical significance - utilize for instance the link 10.1371/journal.pone.0191172.

AA comment 1.d:

As the article suggested by the reviewer and others reflect, carotid intima media thickness as a marker for subclinical atherosclerosis and cardiovascular events has been penalized from its beginning by the heterogeneity of measurement protocols and its lack of reproducibility, which is traduced in a highly variability association. This issue is reflected in the article proposed by the reviewer: "*the range of common CIMT change, compared to CIMT, is very wide both within and between cohorts, indicating that measurement error is a major issue*" and other articles such in Bots' meta-analysis where the correlation range between carotid intima media thickness and significant coronary artery disease was -0.04 to 0.51 (EHJ 2007; 28: 398–406) or in Navqui's et al review (J Am Coll Cardiol Img 2014;7:1025–38) where is also reflected that definition of an abnormal intima media thickness differs between studies; in that sense it seems easier to recognize and measure carotid plaque as it is defined by Manheim Consensus. Secondly, as Lorenz et al reflected there are also biological explanation that is the variability of atherosclerosis development between different territories and, also its complexity. In this sense we agree with Lorenz's et al discussion: "*perhaps an isolated investigation of CIMT is too limited*" and "*the association between plaque and cardiovascular event risk may be closer than between CIMT and risk*". Finally, we believe that we were accurate with our judgments and comments in the sense that current clinical European Guidelines on cardiovascular disease prevention in clinical practice (Eur Heart J. 2016 Aug 1;37(29):2315-2381), published quite recently and followed in our country, supports our arguments considering carotid ultrasound intima media thickness screening for cardiovascular risk assessment is not recommended (Class III level A indication) and considering carotid plaque measurement as a IIb B recommendation. In the same line, the current European guidelines for the management of arterial hypertension

published this year (European Heart Journal (2018) 39, 3021–3104) indicates that between very high risk patients are not include those with increase in carotid intima-media thickness.

Once having established our arguments, we have proceeded to change the discussion to make it closer to the reviewer's view and show how difficult is to evaluate this feature in daily practice.

RV_ 03846820 comment 1.e.: What "carotid plaque" truly means in your case - with a stenosis below 50% (ultrasound-revealed), asymptomatic patients - please, define it transparently.

AA comment 1.e.:

Carotid plaque definition was written in this way to save words. We thought that our definition was clear as in the abstract it is specified that we follow Manheim expert consensus for carotid plaque definition. The reference was added in carotid ultrasonography subsection; moreover, this consensus is also referred in the suggested article mentioned above by the reviewer 10.1371/journal.pone.0191172. We have added in methods section carotid ultrasonography subsection the accurate definition of carotid plaque following reviewer's advice: ***"CP was defined as focal structures encroaching into the arterial lumen of at least 0.5 mm or 50% of the surrounding CIMT value, or demonstrates a thickness >1.5 mm as measured from the intima-lumen interface to the media adventitia interface[21-23]"***

RV comment 1.f.: Should you talk about "adverse events" or about "clinical outcomes"? Please, optimize your definitions in accordance with the common sense and your initial idea.

AA_comment 1.f.:

After a meeting with all the authors we prefer to maintain the term adverse event as it reflects more reliably our initial idea when we planned the research

RV_ 03846820 comment 2: Please, provide the reader with the understanding about your sample size calculation and therefore about your statistical power. Furthermore, there is a room to elaborate your Limitations dramatically. Please, be critical - this is a way for your paper to survive.

AA comment 2:

We did not calculate the sample size. On the one hand, our definition of adverse events (a combined endpoint of all-cause mortality, myocardial infarction and cerebrovascular accident) differs from previous exercise echocardiography studies because carotid disease have been related not only with cardiac events, but also with stroke- Adverse events reported in recent stress echocardiography articles are: all-cause mortality and major adverse cardiac events (Bouzas et al; J Am Coll Cardiol 2009;53:1981–90), myocardial infarction and cardiac death in Metz meta-analysis (J Am Coll Cardiol 2007;49:227–37); nonfatal myocardial infarction and cardiac death or total cardiac events in Makani meta-analisis (J Am Coll Cardiol 2012;60:1393–401); total mortality in Marwick's et al study (Circulation. 2001;103:2566-2571) and all-cause mortality, nonfatal myocardial infarction, and unplanned coronary revascularization or cardiac mortality, nonfatal AMI, and unplanned coronary revascularization or cardiac mortality and AMI in Ahmadvazir's et al study (Ahmadvazir, JACC Cardiovasc Imaging. 2018; 11(2 Pt 1):173-180). On the other hand, there are no data in the scientific

literature about cardiovascular and cerebrovascular events in obese patients with a good prognosis stress echocardiography.

In methods section statistical analysis subsection we have added the next paragraph **"No statistical sample-size calculation was undertaken in this study as this was a pioneering unicentric experience in terms of using carotid ultrasonography in obese patients with good prognosis EE. Furthermore, no published findings from studies with a similar clinical design could be found to enable statistical determination of what sample size of subjects per group is needed to answer the research question.".**

RV_ 03846820 comment 3: Another option is a degree of the obesity and a per cent of the patients with a morbid obesity. How many of them were severely obesed? As you know a degree of the obesity plays the certain role in prediction of the clinical outcomes.

AA comment 3:

In table 1(baseline characteristics) it is reflected that 8 patients (3.5%) had grade 3 obesity (body mass index ≥ 40 Kg/m^2). In the univariate analysis body mass index, expressed as continuous quantitative variable, was not associated with adverse events (hazard ratio 0.93; 95% confidence interval 0.80 – 1.09, p= 0.381). We have performed a new analysis with obesity as a categorical variable (grade 1 to grade 3) we did not fid significant difference between grade 3 obesity (hazard ratio 1.01, 95% confidence interval 0.11 – 9.06, p= 0.933) or grade 2 obesity (hazard ratio 1.05, 95% confidence interval 0.14 – 7.84, p= 0.960) respecting grade 1 obesity.

RV_03846820 comment 4: Methods: what are about medications? Did they take aspirin due to carotid atherosclerosis or by any other reason? Please, elaborate it. It could be nice to have even simple analysis of any associations between medications and outcomes.

AA comment 4:

The population of our study are obese patients with coronary artery disease suspicion. Angina is a clinical diagnosis and treatment was established by physician in the first visit after exercise echocardiography results taking into account not only stress test results but also other conditions such as coronary artery disease pre-test probability, cardiovascular risk factors or carotid disease. For that reason, it is not surprising to find patients with a negative test taking antiplatelet or antianginal agents. In this study medications were removed from adverse events analysis after a debate involving all authors. We think that baseline medications are difficult to maintain during the study (mean follow up time 8.2 ± 2.1 years) and can skew the results because they can be easily added or withdrawal by the different professionals who are in charge of the patient during this long period of time.

RV_03846820 comment 6: Methods: please, explain how your ultrasound analysis was organized in sense of the expert examination - how many investigators were involved, did you re-assess it. Did you have any independent adjudication of both ultrasound data and clinical outcomes? Please, reflect these details in Methods.

AA comment 6:

We reanalysed the stored images by 2 expert imaging cardiologist and a third cardiologist with more than 1000 exercise echocardiographies and carotid ultrasonographies were consulted in case of disagreement, a clinical cardiologist adjudicated adverse events. We remit the reviewer to methods section in page 7 line 1: **"Both EE and carotid ultrasonography stored images were analysed by two imaging-expert cardiologists blinded to the AE. In case of disagreement, a third expert was consulted.".** Moreover in page number 7 line 14 we have added the next paragraph: **"A clinical cardiologist blinded to EE and carotid ultrasonography results adjudicated AE.".**

AUTHORS´ REBUTTAL NOTE – Reviewer´s code: **03465354**

RV_03465354 comment 1: Besides the limitation of this study that the authors state in the Discussion section (retrospective character and one center-based), in my opinion, this study brings additional important knowledge in the field. As a peer reviewer, I do not have any further concerns.

AA_comment:

Thank you for your kind comments about our research

Yours sincerely,

The authors.