Author's response to reviews

Title: Cocoa intake and arterial stiffness in subjects with cardiovascular risk factors.

Authors:

JOSE I RECIO-RODRIGUEZ JIR (donrecio@gmail.com)
MANUEL A GOMEZ-MARCOS MAG (magomez@usal.es)
MARIA C PATINO-ALONSO MCP (carpatino@usal.es)
CRISTINA AGUDO-CONDE CA (cagudoconde@yahoo.es)
EMILIANO RODRIGUEZ-SANCHEZ ER (emilianorodriguezsanchez@yahoo.es)
LUIS GARCIA-ORTIZ LG (Lgarciao@usal.es)

Version: 5 Date: 10 November 2011

Author's response to reviews: see over
Dear Editor of the Nutrition Journal:

Following the suggestions of the reviewers, we enclose a new version of our manuscript entitled: “Cocoa intake and arterial stiffness in subjects with cardiovascular risk factors”, Re: 1184207324580857, together with replies to all the issues raised.

GENERAL COMMENT:

1. The current version of the manuscript has been fully checked by a native English speaker with expertise in scientific translations. A certificate of quality is enclosed.

2. All the changes made in the manuscript (text, tables and figures) are underlined.

3. We included a Conclusions section as the last section of the text. This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance.

4. We include an author's information section for any relevant information about the author(s) that may aid the reader's interpretation of the article, and understand the standpoint of the author(s). This may include details about the authors' qualifications, current positions they hold at institutions or societies, or any other relevant background information.

5. Following the suggestions of the reviewers, we think that the manuscript has improved in terms of understanding and clarity. We therefore think that its interest has increased considerably.
Reviewer: Kiran Ahuja (2)

Major issues:

**Point 1: “Introduction does not provide a good enough rational for conducting this trial”**

We have improved the Introduction, including citations to two recent metaanalyses (Buitrago-Lopez et al. and Khawaja O et al.). Both agree that the mechanisms by which cocoa consumption influences cardiovascular disease remain unclear. They also coincide on the need for further studies before recommending cocoa consumption in both high risk patients and in healthy subjects.

The following has been added to the Introduction (page 3, line 2):

Two recently published metaanalyses coincide that it is unclear whether cocoa consumption is related to reductions in adverse cardiovascular outcomes. The relationship between cocoa consumption and arterial stiffness has only been studied in healthy individuals without cardiovascular risk factors.

**Point 2: “Rationale not provided for categorising the chocolate intake into <4.29g/day or >4.29g/d consumption. Most other studies discuss the health benefits of chocolate/cocoa at 6g or higher consumption per day”**

The questionnaire used for documenting cocoa consumption is a semiquantitative instrument validated in Spain by the University of Navarre [12]. This type of questionnaire does not allow us to use the variable “cocoa consumption” as a pure continuous variable. As a result, we decided to express cocoa consumption following the reply options of the questionnaire: Never, 1-3 servings/month, weekly, 2-4 servings/week, 5-6 servings/week, daily, 2-3 servings/day, 4-6 servings/day, more than 6 servings/day.

We have taken low consumption to represent the intake of ≤ 1 serving/week. This frequency estimates a cocoa intake of 4.29 g/day. The next category (2-4 servings/week) estimates a consumption of 12.86 g/day. Accordingly, no subject presented an estimated consumption of 6 g/day. This is the reason for categorizing consumption into over 4.29 g/day or less.

Under Methods, we have modified the methodology used to document cocoa consumption, which now reads as follows (page 4, line 1):

**Information about the frequency of cocoa intake and all other foods was collected using the food frequency questionnaire of the University of Navarre, validated for Spain [9]. This questionnaire categorizes cocoa consumption as follows: Never, 1-3**
servings/month, weekly, 2-4 servings/week, 5-6 servings/week, daily, 2-3 servings/day, 4-6 servings/day, more than 6 servings/day.

Cocoa intake was determined as follows: the average daily consumption (weight) of cocoa-containing foods and beverages was multiplied by their individual percentage of cocoa contents, which were derived from food labels and from published data. Cocoa intake from individual foods was summed to yield the actual intake of cocoa in grams per day for each subject.

We posteriorly divided the subjects according to estimated cocoa consumption. Low cocoa consumption was taken to represent the intake of $\leq 1$ serving/week.

In Table 1 we have added the following information in the designation of each group: low consumption ($\leq 1$ serving/week) and high consumption ($> 1$ serving/week)

Point 3: “Median chocolate intake has been shown to be 2g/day. This means most of the people in the study have low chocolate intake making the data skewed to no/low chocolate intake. This may possibly impact on the statistics used. I am not an expert in statistics but I wonder if it would have been better to analyse the data (chocolate intake) as a continuous variable in the linear regression model than as a categorical variable”

The questionnaire used for documenting cocoa consumption is a semiquantitative instrument. This type of questionnaire does not allow use of the variable “cocoa consumption” as a pure continuous variable. As a result, we decided to express cocoa consumption following the reply options of the questionnaire: Never, 1-3 servings/month, weekly, 2-4 servings/week, 5-6 servings/week, daily, 2-3 servings/day, 4-6 servings/day, more than 6 servings/day.

We also estimated the mean total cocoa consumption of the sample (4.30 ± 9.08 g/day) and of those subjects that consumed some cocoa (mean 7.74 ± 11.04 g/day), as well as the median (2 g/day).

Methods now reads as follows (page 4, line 1):

Information about the frequency of cocoa intake and all other foods was collected using the food frequency questionnaire of the University of Navarre, validated for Spain [9]. This questionnaire categorizes cocoa consumption as follows: Never, 1-3 servings/month, weekly, 2-4 servings/week, 5-6 servings/week, daily, 2-3 servings/day, 4-6 servings/day, more than 6 servings/day.

We posteriorly divided the subjects according to estimated cocoa consumption. Low cocoa consumption was taken to represent the intake of $\leq 1$ serving/week.

We have added the following text under Results (page 6, line 2):

Mean cocoa consumption of the global sample was 4.30 ± 9.08 g/day, with an intake among those who consumed some cocoa of 7.74 ± 11.04 g/day, and a median value of 2 g/day.
Point 4: In the results the authors suggest a higher PWV in non-chocolate consumers but in discussion they say that chocolate intake is not associated with vascular stiffness. I would like to see reasoning for coming to this conclusion.

PWV showed a significant difference between the non-consumers (9.22 m/sec) and the high consumption group (8.47 m/sec) (p=0.039). However, on adjusting for age, gender, the presence of diabetes, systolic blood pressure and antihypertensive and lipid-lowering drug use, this difference disappeared. We therefore concluded that cocoa intake is not associated with vascular stiffness in subjects with cardiovascular risk factors.

The following has been added under Results (page 6, line 15):

PWV showed a significant difference between the non-consumers (9.22 m/sec) and the high consumption group (8.47 m/sec) (p=0.039). However, on adjusting for age, gender, the presence of diabetes, systolic blood pressure and antihypertensive and lipid-lowering drug use, this difference disappeared.

Point 5: “The authors do not report if they separated the data into normal and dark chocolate or cocoa content. Most literature discusses the data in terms of cocoa content.”

We have changed the term chocolate to cocoa throughout the manuscript, for improved clarity.

The title has been modified and now reads as follows:
Cocoa intake and arterial stiffness in subjects with cardiovascular risk factors.

The following has been added under Methods (page 4, line 5):
Cocoa intake was determined as follows: the average daily consumption (weight) of cocoa-containing foods and beverages was multiplied by their individual percentage of cocoa contents, which were derived from food labels and from published data. Cocoa intake from individual foods was summed to yield the actual intake of cocoa in grams per day for each subject.

Point 6: “Discussion is minimal. What is provided is the repetition of results and results of other studies. There is no discussion as why the authors think the results of this study are different from other studies”.

Following the recommendations of the reviewer, we have modified the Discussion, which now reads as follows (page 7, line 6):

Our results differ from those reported by Vlachopoulos et al.[7], who related regular cocoa intake to decreased aortic stiffness and improved central hemodynamics. This study was conducted in healthy subjects, while our population was more heterogeneous and consisted of subjects with some cardiovascular risk factor (hypertension, diabetes, or dyslipidemia), since these are the subjects most commonly attended in the primary care setting. These risk factors can influence the measures used to estimate arterial stiffness. In addition, many of the patients were receiving antihypertensive medication
(53.80% among the non-consumers, 45.30% in the low consumption group, and 37.10% in the high consumption group) and lipid-lowering drugs, which may influence the results obtained. However, the interest of the study is that no previous evaluations have been made of the relationship between cocoa consumption and arterial stiffness in subjects of this kind. In contrast to reports by Djousse L and Lewis JR [9, 10], no significant relationship was found in our population between C-IMT and cocoa intake. The most likely explanation for this discrepancy is the proportion of subjects with antihypertensive and lipid-lowering drugs. This percentage was lower in the high consumption group than among the non-consumers.

Minor issues:

**Point 1: “No references are given for the technique use. No information on the equipment (company etc)”**

We have added the following under Methods (page 4, line 15):

Central blood pressure (CBP) and central and peripheral augmentation index (CAIx, PAIx) were estimated using the SphygmoCor System (AtCor Medical Pty Ltd., Head Office, West Ryde, Australia).

We have added the following citation in calculation of the ambulatory arterial stiffness index:


In the section relating to pulse wave velocity, we have added the following (page 4, line 22):

Using the SphygmoCor System (Vx pulse wave velocity), PWV was measured with the patient in the supine position, estimating the delay in pulse wave at carotid and femoral level as compared to the electrocardiogram wave.

In the section relating to estimation of C-IMT, we have added the following (page 5, line 6):

A Sonosite Micromax ultrasound device (Sonosite Inc., Bothell, Washington, USA) paired with a 5-10 MHz multifrequency high-resolution linear transducer with Sonocal software was used for performing automatic measurements of IMT, in order to optimize reproducibility.

**Point 2: “Need improvement in English.”**

The current version of the manuscript has been fully checked by a native English speaker with expertise in scientific translations. A certificate of quality is enclosed.

**Point 3: “I wonder what do the authors mean by ‘black’ chocolate in Discussion”**

We have removed the word “black”, in order to avoid interferences in the text.
Reviewer: Kimon Stamatelopoulos (1)

Major comments:

Point 1: “Diabetes is considered a CAD equivalent conferring very high cv risk as compared to other cv risk factors. Therefore it is not safe to jump to conclusions when using mixed populations with and without DM in primary prevention settings. This problem is augmented when assessing dietary interventions affecting glucose control. It is not surprising that most patients in the group of no chocolate consumption, DM was highly prevalent (>30%) since chocolate is not recommended in a diabetic diet. On the other hand DM patients who reported increased chocolate use were probably not compliant to other medical recommendations as well resulting in a higher overall cv risk profile. Thus including DM patients in this kind of study introduces several biases unrelated to chocolate effects per se. The data should be reanalyzed excluding patients with DM”

We have re-analyzed the data, excluding the diabetics from each group, without any modification of the results obtained. Indeed, the difference in PWV between the high consumer group and the non-consumers disappeared in this new analysis. (See attached table of results without diabetics. (See ANNEX)). In the table of results we have added the percentage subjects using antihypertensive and lipid-lowering drugs.

We have studied individuals with risk factors because these are the subjects most commonly seen in the primary care setting. These risk factors can influence the measures used to estimate arterial stiffness. In addition, many of the patients were receiving antihypertensive medication (53.80% among the non-consumers, 45.30% in the low consumption group, and 37.10% in the high consumption group) and lipid-lowering drugs, which may influence the results obtained. However, the interest of the study is that no previous evaluations have been made of the relationship between cocoa consumption and arterial stiffness in subjects of this kind. The conclusion is that possibly cocoa consumption in subjects of this kind does not imply improvement in the arterial stiffness values – the latter being more influenced by antihypertensive and lipid-lowering drug use.

This is surely also the reason for the results obtained in reference to the relationship between IMT and cocoa consumption.

The following phrases have been added under Results (page 6, line 10 y 21):
We re-analyzed the data, excluding the diabetics, without any modification of the results or conclusions obtained.

The proportion of patients treated with drugs was greater among the non-consumers of cocoa than in the high consumption group, referred to both the antihypertensive agents (53.80% vs 37.80%) and the lipid-lowering drugs (37.10% vs 15.70%).

The Discussion has been worded as follows (page 7,line 6):
Our results differ from those reported by Vlachopoulos et al.,[7] who related regular cocoa intake to decreased aortic stiffness and improved central hemodynamics. This study was conducted in healthy subjects, while our population was more heterogeneous and consisted of subjects with some cardiovascular risk factor (hypertension, diabetes, or dyslipidemia), since these are the subjects most commonly attended in the primary care setting. These risk factors can influence the measures used to estimate arterial stiffness. In addition, many of the patients were receiving antihypertensive medication (53.80% among the non-consumers, 45.30% in the low consumption group, and 37.10% in the high consumption group) and lipid-lowering drugs, which may influence the results obtained. However, the interest of the study is that no previous evaluations have been made of the relationship between cocoa consumption and arterial stiffness in subjects of this kind. In contrast to reports by Djousse and Lewis [9, 10], no significant relationship was found in our population between C-IMT and cocoa intake. The most likely explanation for this discrepancy is the proportion of subjects with antihypertensive and lipid-lowering drugs. This percentage was lower in the high consumption group than among the non-consumers.

The final conclusion has been worded as follows (page 8, line 4):

In conclusion, in subjects with some cardiovascular risk factors, cocoa consumption does not imply improvement in the arterial stiffness values.

Point 2: “There are also other differences between subgroups which further prevents correct interpretation of these results. Age and gender differences are quite pronounced and I am not certain if statistical adjustment is sufficient for differences of this magnitude because populations may not be comparable. Where the participants consecutively recruited? Moreover, adjustments for BP parameters and particularly those differing among groups should be performed since arterial stiffening is mainly affected by age and BP”

The patients were recruited in the primary care centers. The results were adjusted for variables that could be related to arterial stiffness, and for variables showing important inter-group differences. Thus, we adjusted the results for age, gender, diabetes, systolic blood pressure and antihypertensive and lipid-lowering drugs, with no resulting changes.

The section referred to the statistical analysis now reads as follows (page 5, line 21):
Results were adjusted for age and gender, systolic blood pressure, diabetes and the presence of medical treatment (antihypertensive and lipid-lowering drugs), based on analysis of covariance (ANCOVA).

Point 3: “Medical treatment and particularly antihypertensive and hypolipidemic drugs should be reported and included in the multivariate analysis”

In the table of results we have added the percentage subjects using antihypertensive and lipid-lowering drugs. The following phrase has been added under Results (page 6, line 10):
The proportion of patients treated with drugs was greater among the non-consumers of cocoa than in the high consumption group, referred to both the antihypertensive agents (53.80% vs 37.80%) and the lipid-lowering drugs (37.10% vs 15.70%).

We have adjusted the results for age, gender, diabetes, systolic blood pressure and antihypertensive and lipid-lowering drugs, with no resulting changes. In addition, we have added the following text to the statistical analysis section (page 5, line 21):

Results were adjusted for age and gender, systolic blood pressure, diabetes and the presence of medical treatment (antihypertensive and lipid-lowering drugs), based on analysis of covariance (ANCOVA).

Point 4: “The sample size of the subgroup with high chocolate consumption is marginally not adequate to assess differences in PWV. Since the authors report negative results, it is particularly important for this study to provide sufficient power. The SD for the PWV values used in their sample size calculations was too high. It is possible that SD would be smaller (and therefore a smaller sample would be necessary) if DM patients were excluded since the study group would become more homogeneous”

We have estimated the power of the contrast as 81% for detecting differences in PWV between high consumers and non-consumers, and as 42% between high consumers and low consumers.

The SD without diabetic patients is 2.06, as a result of which the sample size estimation would decrease from 279 to 267, i.e., only 12 subjects less, and the diabetics total are 89.

We have re-analyzed the data, excluding the diabetics from each group, without any modification of the results obtained. Indeed, the difference in PWV between the high consumer group and the non-consumers disappeared in this new analysis. Likewise, on adjusting for age, gender, the presence of diabetes, systolic blood pressure and antihypertensive and lipid-lowering drug use, the results remained without change. For these reasons we have maintained all the subjects in the manuscript – unless both reviewers decide it is best to suppress the diabetics, in which case Table 1 would be modified, with adaptation of the rest of the results. Table 1 without the diabetics is attached at the end of the replies to the reviewers.

Minor comments:

Point 1: Please provide in summary the methods and materials used to measure the end-points of this study.

We have added the following under Methods (page 4, line 15):

Central blood pressure (CBP) and central and peripheral augmentation index (CAIx, PAIx) were estimated using the SphygmoCor System (AtCor Medical Pty Ltd., Head Office, West Ryde, Australia).
We have added the following citation in calculation of the ambulatory arterial stiffness index:

In the section relating to pulse wave velocity, we have added the following (page 4, line 22):
Using the SphygmoCor System (Vx pulse wave velocity), PWV was measured with the patient in the supine position, estimating the delay in pulse wave at carotid and femoral level as compared to the electrocardiogram wave.

In the section relating to estimation of C-IMT, we have added the following (page 5, line 6):
A Sonosite Micromax ultrasound device (Sonosite Inc., Bothell, Washington, USA) paired with a 5-10 MHz multifrequency high-resolution linear transducer with Sonocal software was used for performing automatic measurements of IMT, in order to optimize reproducibility.

**Point 2: “At some points the text in results is inconsistent with the provided tables and Figures”**

Figure 1 represents the values corresponding to PWV, AASI, C-IMT and PAIX in the different cocoa consumption groups, after adjusting for age and gender, diabetes, systolic blood pressure and antihypertensive and lipid-lowering drugs.

**Point 3: “Percentages in the table should be presented as a proportion within each group separately”**

The table has been corrected as indicated.

**Point 4: Quality of written English: Needs some language corrections before being published**

The current version of the manuscript has been fully checked by a native English speaker with expertise in scientific translations. A certificate of quality is enclosed.
ANNEX: Table 1 reporting the results after excluding the diabetic subjects.

<table>
<thead>
<tr>
<th>Table 1. General demographic and clinical characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Non- consumption (n=98)</td>
</tr>
<tr>
<td>Age 55.33±11.82</td>
</tr>
<tr>
<td>Male (n, %) 54 (55.1)</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
</tr>
<tr>
<td>Hypertension (n, %) 78 (79.6)</td>
</tr>
<tr>
<td>Obesity (BMI&gt;30) (n, %) 26 (26.5)</td>
</tr>
<tr>
<td>Dyslipidemia (n, %) 79 (80.6)</td>
</tr>
<tr>
<td>Smoking (n, %) 24 (24.5)</td>
</tr>
<tr>
<td>BMI (Kg/m²) 27.88±3.79</td>
</tr>
<tr>
<td>Waist circumference (cm) 95.11±11.57</td>
</tr>
<tr>
<td>Serum glucose (mg/dL) 87.90±11.23</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL) 212.00±39.10</td>
</tr>
<tr>
<td>Cardiovascular risk (Score) 3.01±3.58</td>
</tr>
<tr>
<td>Cardiovascular risk (D’Agostino) 16.65±11.61</td>
</tr>
<tr>
<td>Office SBP (mmHg) 140.15±16.71</td>
</tr>
<tr>
<td>Office DBP (mmHg) 87.71±10.22</td>
</tr>
<tr>
<td>Peripheral pulse pressure (mmHg) 52.44±13.00</td>
</tr>
<tr>
<td>Central SBP (mmHg) 133.65±18.88</td>
</tr>
<tr>
<td>Central DBP (mmHg) 89.89±11.08</td>
</tr>
<tr>
<td>Central pulse pressure (mmHg) 43.77±15.26</td>
</tr>
<tr>
<td>24 h SBP (mmHg) 125.77±12.79</td>
</tr>
<tr>
<td>24 h DBP (mmHg) 78.21±9.47</td>
</tr>
<tr>
<td>24 h Pulse pressure (mmHg) 47.55±9.36</td>
</tr>
<tr>
<td>PWV (m/s) 8.69±1.86</td>
</tr>
<tr>
<td>C-IMT mean (mm) 0.73±0.10</td>
</tr>
<tr>
<td>C-IMT maximum (mm) 0.89±0.12</td>
</tr>
<tr>
<td>CAIx 30.30±11.34</td>
</tr>
<tr>
<td>PAIx 94.60±22.74</td>
</tr>
<tr>
<td>Day AASI 0.37±0.06</td>
</tr>
<tr>
<td>Night AASI 0.37±0.15</td>
</tr>
<tr>
<td>ABI 1.08±0.10</td>
</tr>
<tr>
<td>Total energy (Kcal/day) 2331.46±710.65</td>
</tr>
<tr>
<td>Carbohydrates (g/day) 263.26±90.48</td>
</tr>
<tr>
<td>Protein (g/day) 101.62±32.04</td>
</tr>
<tr>
<td>Total fat (g/day) 88.00±33.92</td>
</tr>
</tbody>
</table>

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, IMT: Intima-media thickness, PWV: Pulse wave velocity, C-IMT: Carotid intima-media thickness, CAIx: Central augmentation index, PAIx: Peripheral augmentation index, AASI: Ambulatory arterial stiffness index, ABI: ankle-brachial index

Data for qualitative variables are expressed as n (%) and quantitative variables as mean ± standard deviation

p: statistically significant differences (p < 0.05)