

## PEER REVIEW HISTORY

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## ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Vascular and cognitive effects of cocoa-rich chocolate in postmenopausal women: A study protocol for a randomized clinical trial
<b>AUTHORS</b>	Garcia-Yu, Irene; Garcia-Ortiz, Luis; Gomez-Marcos, Manuel; Alonso-Dominguez, Rosario; González-Sánchez, J; Mora-Simon, Sara; González-Manzano, Susana; Rodriguez-Sanchez, Emiliano; Maderuelo-Fernandez, Jose; Recio-Rodriguez, Jose

## VERSION 1 – REVIEW

<b>REVIEWER</b>	Isabella Sudano University Heart Center Cardiology University Hospital Zürich, Switzerland
<b>REVIEW RETURNED</b>	22-May-2018

<b>GENERAL COMMENTS</b>	<p>The author should add informations about the allowed consumption of the study chocolate: will be the participant allowed to melt the chocolate with milk?</p> <p>Will the author evaluate epicathechins in plasma? In my opinion this would be needed for a correlation with the endpoint.</p> <p>The effect of chocolate, cocoa and epicathechins on cognitive function was already topic of previous studies. The actual available literature about chocolate and epicathechins is not adequately cited: I suggest to revised the literature adding for example such articles:</p> <p>Recommending flavanols and procyanidins for cardiovascular health: Revisited. Ottaviani JI, Heiss C, Spencer JPE, Kelm M, Schroeter H. Mol Aspects Med. 2018 Feb 23. Brachial and Cerebrovascular Functions Are Enhanced in Postmenopausal Women after Ingestion of Chocolate with a High Concentration of Cocoa. Marsh CE, Carter HH, Guelfi KJ, Smith KJ, Pike KE, Naylor LH, Green DJ. J Nutr. 2017 Sep;147(9):1686-1692. Enhancing Human Cognition with Cocoa Flavonoids. Socci V, Tempesta D, Desideri G, De Gennaro L, Ferrara M. Front Nutr. 2017 May 16;4:19. Absorption, metabolism, distribution and excretion of (-)-epicatechin: A review of recent findings. Borges G, Ottaviani JI, van der Hooft JJJ, Schroeter H, Crozier A. Mol Aspects Med. 2017 Nov 9. pii: S0098-2997(17)30143-7. Chocolate intake is associated with better cognitive function: The Maine-Syracuse Longitudinal Study.</p>
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	<p>Crichton GE, Elias MF, Alkerwi A. Appetite. 2016 May 1;100:126-32.</p> <p>Cocoa, Blood Pressure, and Vascular Function.</p> <p>Ludovici V, Barthelmes J, Nägele MP, Enseleit F, Ferri C, Flammer AJ, Ruschitzka F, Sudano I. Front Nutr. 2017 Aug 2;4:36.</p> <p>Effect of cocoa on blood pressure.</p> <p>Ried K, Fakler P, Stocks NP. Cochrane Database Syst Rev. 2017 Apr 25;4:CD008893.</p> <p>High-flavonoid intake induces cognitive improvements linked to changes in serum brain-derived neurotrophic factor: Two randomised, controlled trials.</p> <p>Neshatdoust S, Saunders C, Castle SM, Vauzour D, Williams C, Butler L, Lovegrove JA, Spencer JP. Nutr Healthy Aging. 2016 Oct 27;4(1):81-93.</p> <p>Habitual cocoa intake reduces arterial stiffness in postmenopausal women regardless of intake frequency: a randomized parallel-group study.</p> <p>Okamoto T, Kobayashi R, Natsume M, Nakazato K. Clin Interv Aging. 2016 Nov 14;11:1645-1652.</p> <p>Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women.</p> <p>Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs DR Jr. Am J Clin Nutr. 2007 Mar;85(3):895-909.</p>
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<b>REVIEWER</b>	Channa Marsh The University of Western Australia, Australia.
<b>REVIEW RETURNED</b>	04-Jun-2018

<b>GENERAL COMMENTS</b>	<p>This has the potential to be very influential research for postmenopausal women and I look forward to hearing about it in the future, however there are a few minor points that need clarification in your protocol.</p> <p>Important to state whether there is a standardised protocol prior to each laboratory visit. For example, refraining from polyphenol containing foods, caffeine, alcohol and physical activity 24hrs prior to each laboratory visit; arrive fasted (I'd assume so as you have fasted glucose as one of your measures); standardised time of day for testing within-participants.</p> <p>Exclusion criteria: Consider excluding those who currently, or have in the past 12 months, smoke/d cigarettes as they are known to influence your outcome measures, especially vascular function and blood pressure.</p> <p>Chocolate condition:</p> <ul style="list-style-type: none"> <li>• Provide some justification for such a small amount of chocolate (10g per day) to be used.</li> <li>• You stated the chocolate will be consumed at the same time each day along with other eating instructions. State any recommendations for participants on the food and liquids consumed around this time to maximise the absorption of the cocoa polyphenols.</li> </ul> <p>Measurements:</p> <ul style="list-style-type: none"> <li>• State the laboratory environment standardisation for this blood pressure and vascular function assessments. I.e. in a stimulating-</li> </ul>
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	<p>free environment that limits surrounding noise; rest period, lighting and temperature standardised if possible.</p> <ul style="list-style-type: none"> <li>• It would be recommended to have a familiarisation session for your cognitive tests prior to baseline measurements to ensure limiting a learning effect over the subsequent 3 and 6 month testing periods.</li> <li>• In regards to body composition, postmenopausal women are at an increased risk of weight gain, particularly adipose tissue gain, due to the reduction of endogenous estrogen associated with the menopause transition. Therefore, assessing fat mass and body fat percentage would be the primary outcomes for your body composition measurements. Although I am not fully across the board with the literature surrounding the Inbody 230 Monitor in assessing body composition, the literature that I have read suggest that although lean body mass is accurately measured, body fat mass and body fat percentage are considerably underestimated by this machine. Is this equipment more accurate in repeated measures changes, rather than absolute values for fat mass and percentage? Have you considered using another body composition method in conjunction with this method such as DXA, or even skin folds?</li> </ul> <p>Article to include: Marsh, et al, 2017. Brachial and cerebrovascular functions are enhanced in postmenopausal women after ingestion of chocolate with a high concentration of cocoa. Journal of Nutrition. doi: <a href="https://doi.org/10.3945/jn.117.250225">https://doi.org/10.3945/jn.117.250225</a></p>
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## VERSION 1 – AUTHOR RESPONSE

**Reviewer: 1**

**Reviewer Name: Isabella Sudano**

**Institution and Country: University Heart Center Cardiology University Hospital Zürich, Switzerland**

**Competing Interests: None declared**

**Answer:**

First of all, thank the reviewer's comments because they are very successful and improve the work significantly.

**The author should add informations about the allowed consumption of the study chocolate: will be the participant allowed to melt the chocolate with milk?**

**Answer:**

There are few studies that analyze the possible relationship between food intake and the absorption of cocoa polyphenols and, in addition, the results obtained are inconclusive. In the study by Keogh et al. [1] the milk slightly accelerated the absorption of polyphenols, although it did not influence the average

concentration reached. Likewise, van het Hof et al. [2] concluded that the addition of milk to green tea or black tea did not affect plasma catechin levels. Wang et al. [3] observed in a study, in which chocolate was consumed with 40 g of bread, that the plasma concentration of epicatechin was higher than the average value achieved in a previous study conducted by the same group in which the subjects did not consume any additional food (bread) until 4 h after the chocolate intake. However, these authors conclude that the influence of the bread on the bioavailability of epicatechin from chocolate must be determined in future studies.

Here are some of the recommendations that are given to the participants:

- The chocolate in the studio has 99% cocoa, so its flavor is slightly more bitter and less sweet than the chocolate that is usually consumed. According to daily consumption, you will get used to the taste and sensations you experience.
- It is recommended to consume the chocolate every day at about the same time.
- To improve the tasting you can consume the chocolate in small pieces leaving them unmated in the mouth without chewing, but refrain to melt the chocolate with milk.

We have introduced some of these instructions in the text (methodology section) (Pag 8, line 208):

Participants will also be given instructions on eating and keeping the product, with the recommendations, for example, that the chocolate can be consumed in small pieces leaving them unmated in the mouth, without chewing them. In addition, a series of recommendations will be given remembering the organoleptic characteristics of the product, as well as the recommendations of trying to consume the product at the same time or refrain from ingesting it dissolved in milk.

**Will the author evaluate epicatechins in plasma? In my opinion this would be needed for a correlation with the endpoint.**

Answer:

We agree with the reviewer on the relevance of the bioavailability of polyphenols for the determination of the possible effects on health, so it would be very interesting to evaluate epicatechins in plasma. Although this is not included in the main objectives of this study or in its funding, we plan to carry out a sub-study that will include a sub-sample of women from the ECCAMP study who will try to identify phenolic metabolites in urine to evaluate its relationship with possible vascular effects and cognitive function. However, we have the daily amount of epicatechins that will contribute the 10 g of chocolate added to their usual diet in the intervention group (Table 1), which will be  $2,610 \pm 0.075$  mg / g.

**The effect of chocolate, cocoa and epicatechins on cognitive function was already topic of previous studies. The actual available literature about chocolate and epicatechins is not adequately cited: I suggest to revised the literature adding for example such articles:**

**Recommending flavanols and procyanidins for cardiovascular health. Ottaviani JI, Heiss C, Spencer JPE, Kelm M, Schroeter H. Mol Aspects Med. 2018 Feb 23.**

**Brachial and Cerebrovascular Functions Are Enhanced in Postmenopausal Women after Ingestion of Chocolate with a High Concentration of Cocoa.** Marsh CE, Carter HH, Guelfi KJ, Smith KJ, Pike KE, Naylor LH, Green DJ. *J Nutr.* 2017 Sep;147(9):1686-1692.

**Enhancing Human Cognition with Cocoa Flavonoids.** Socci V, Tempesta D, Desideri G, De Gennaro L, Ferrara M. *Front Nutr.* 2017 May 16;4:19.

**Absorption, metabolism, distribution and excretion of (-)-epicatechin: A review of recent findings.** Borges G, Ottaviani JI, van der Hooft JJJ, Schroeter H, Crozier A. *Mol Aspects Med.* 2017 Nov 9. pii: S0098-2997(17)30143-7.

**Chocolate intake is associated with better cognitive function: The Maine-Syracuse Longitudinal Study.** Crichton GE, Elias MF, Alkerwi A. *Appetite.* 2016 May 1;100:126-32.

**Cocoa, Blood Pressure, and Vascular Function.** Ludovici V, Barthelmes J, Nägele MP, Enseleit F, Ferri C, Flammer AJ, Ruschitzka F, Sudano I. *Front Nutr.* 2017 Aug 2;4:36.

**Effect of cocoa on blood pressure.** Ried K, Fakler P, Stocks NP. *Cochrane Database Syst Rev.* 2017 Apr 25;4:CD008893.

**High-flavonoid intake induces cognitive improvements linked to changes in serum brain-derived neurotrophic factor: Two randomised, controlled trials.** Neshatdoust S, Saunders C, Castle SM, Vauzour D, Williams C, Butler L, Lovegrove JA, Spencer JP. *Nutr Healthy Aging.* 2016 Oct 27;4(1):81-93.

**Habitual cocoa intake reduces arterial stiffness in postmenopausal women regardless of intake frequency: a randomized parallel-group study.** Okamoto T, Kobayashi R, Natsume M, Nakazato K. *Clin Interv Aging.* 2016 Nov 14; 11:1645-1652.

**Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women.** Mink PJ, Scrafford CG, Barraj LM, Harnack L, Hong CP, Nettleton JA, Jacobs DR Jr. *Am J Clin Nutr.* 2007 Mar;85(3):895-909.

Answer:

We have reviewed the literature on this topic and we have added the articles pointed out by the reviewer, as well as some comments in the introduction section that is now read as follows (Pag 3, line 67):

## INTRODUCTION

Polyphenols are bioactive compounds found in many plants, fruits and vegetables. The beneficial effects on human health associated with the consumption of a diet rich in polyphenols has generated great scientific interest in these substances [4-6]. The action of polyphenols is based on their antioxidant capacity through the uptake of free radicals, the chelation of metals with redox properties and the modulation and inhibition of enzymatic activity [7].

The most abundant polyphenols in cocoa are flavonoids, which have been linked to a protective effect against cardiovascular disease, decreasing the risk of cardiovascular morbidity and mortality and favouring the prevention of other chronic diseases such as diabetes mellitus type 2 [4-6, 8-10]. The ability to reduce cardiovascular risk could be due to an improvement in the elements that define metabolic syndrome, the improvement of vascular endothelial dysfunction, insulin resistance and the inhibition of platelet activation and aggregation [11, 12]. However, although current evidence suggests that polyphenols produce an improvement in cardiovascular health, it is not enough to determine the minimum amount of intake necessary to achieve health benefits. [13].

**Cocoa polyphenols and blood pressure:** The effect of consuming polyphenols present in chocolate on the blood pressure (BP) statistics of healthy individuals is not clear. Cocoa consumption has been associated with an improvement in endothelial function and a decrease in blood pressure in both healthy subjects and those with risk factors and cardiovascular diseases [14, 15]. Some studies have observed a dose-dependent relationship between cocoa intake and clinical BP, with higher consumption equated to lower blood pressure and better vascular function [16, 17]. Conversely, other research has not obtained significant changes in these parameters related to the supplementation of cocoa or pure polyphenols such as epicatechin or quercetin [18, 19].

Endothelial dysfunction in postmenopausal women causes changes that favour the development of cardiovascular risk factors and atherosclerosis, which lead to the appearance and maintenance of hypertension [20, 21]. A decrease in BP has been observed in this group after daily consumption of cocoa with a flavonol content of 40.12 mg. Below this level, however, no changes have been observed [22].

**Cocoa polyphenols and vascular function:** Among healthy individuals as well as postmenopausal women, the consumption of polyphenols present in cocoa has been associated with a dose-dependent improvement of vascular function, in particular of arterial stiffness measured by pulse wave speed [16, 17, 22]. One of these studies also suggests that the reduction in arterial stiffness observed in postmenopausal women after consumption of cocoa is independent of the frequency of the intake [22]. However, this relationship is not evident in people with mild hypertension when cardio-ankle vascular index (CAVI) is used as a measure of arterial stiffness [23].

There is also evidence of the influence of these polyphenols in reducing the augmentation index (AIx). The study by West et al [24], involving subjects with excess weight and moderate obesity, concludes that the treatment with dark chocolate decreases AIx in women, although it seems that this association may affect more the elasticity of the large arteries, especially in subjects with obesity and diabetes mellitus type 2 [25].

**Cocoa polyphenols and cognitive performance:** There is evidence to suggest that chocolate rich in polyphenols may be beneficial for cognitive performance and state since it improves mental processing speed and attenuates the increase of mental fatigue among healthy young adults [26, 27]. An improvement in cognitive performance among older age groups after eating chocolate has also been observed [28] and especially in subjects with higher risk of cardiovascular disease [29]. Several studies also show that polyphenol-rich chocolate causes an improvement in executive function and categorical



fluency [30], in working memory [31, 32], and a slowing of mental fatigue [33] and also that a higher frequency of chocolate consumption has been associated with better cognitive function [32]. Furthermore, a positive influence of cocoa polyphenols on physiological processes has been reported, with a neuroprotective effect [34] and improved cognitive performance [35]. In this regard, it has been suggested that the brain-derived neurotrophic factor (BDNF) may play a role in the cognitive enhancement induced by the flavonoids [36]. Favorable effects on cerebrovascular function have also been observed in postmenopausal women after consumption of chocolate with high concentration of cocoa [37].

**Cocoa polyphenols and quality of life:** The quality of life linked to health is represented by the individual's perception of well-being in various aspects of life, including physical and mental aspects. The effect of chocolate and polyphenols on the quality of life has scarcely been studied, with not a great deal of evidence available and even less of a conclusive nature. In a study conducted among healthy people, where regular consumption of chocolate was recorded over a year, no evidence was found of a clear association between chocolate intake and the physical or mental components of quality of life [38]. Nevertheless, it has been observed that the consumption of dark chocolate may be beneficial for the quality of life of women with fibromyalgia [39].

**Cocoa polyphenols and body composition:** The menopause period leads to various changes in the body composition of women [40]. Regarding the connection between cocoa polyphenols and body composition, results diverge. Some clinical trials involving healthy people and overweight or obese patients have not reported significant differences that link chocolate consumption to anthropometric measures [19, 23, 24, 41]. Other studies indicate that chocolate consumption may have positive effects on body composition in adolescents [42], patients with diabetes [43] or women with obesity [44]. Two recent systematic reviews also indicate that eating chocolate is associated with reduced body mass index (BMI) and waist circumference [45, 46], and one of them also concludes that the amount and the length of time during which it is eaten play a key role in these beneficial effects [46]. Conversely, other studies such as that carried out with the cohort of the Atherosclerosis Risk in Communities (ARIC) study have observed a dose-dependent increase in weight after habitual chocolate consumption [47].

In sum, the polyphenols present in chocolate seem to have a positive effect on BP, vascular function, cognitive performance and quality of life, especially in populations with increased cardiovascular risk such as postmenopausal women [48]. However, the conflicting results obtained in different studies suggest that the real contribution of these compounds to health and the underlying mechanisms remain unclear. Moreover, most of these studies have used preparations with high concentrations of polyphenols that are usually not present in the normal diet.

This study aims to evaluate the effect of adding a daily amount of 10 g of chocolate high in cocoa content (99%) and polyphenols to the normal diet on blood pressure, vascular function, cognitive performance, quality of life and body composition in postmenopausal women.

**Reviewer: 2**

**Reviewer Name: Channa Marsh**

**Institution and Country: The University of Western Australia, Australia.**

**Competing Interests: None declared**

**This has the potential to be very influential research for postmenopausal women and I look forward to hearing about it in the future, however there are a few minor points that need clarification in your protocol.**

Answer:

First, to thank the reviewer for the contributions that are very valuable and improve notably the protocol document.

**Important to state whether there is a standardised protocol prior to each laboratory visit. For example, refraining from polyphenol containing foods, caffeine, alcohol and physical activity 24hrs prior to each laboratory visit; arrive fasted (I'd assume so as you have fasted glucose as one of your measures); standardised time of day for testing within-participants.**

Answer:

In effect, there is a standardized protocol prior to each visit. We have written a summary of this protocol in the methodology section (Pag 13, line 334):

The evaluation visits will be made in the morning between 8:00 and 10:00 a.m. Each participant will be informed prior to the visit to go fasting for at least 12 hours, having avoided the 24 hours prior to visiting the consumption of polyphenol-rich foods, including cocoa, chocolate, apples, and red wine as well as alcoholic drinks or the performance of programmed physical activity. All evaluation visits, including blood pressure measurements and evaluations of vascular function, will be carried out in a room with conditions of lighting and temperature standardized, recommending that patients attend the appointment with a prior rest of at least 8- 10 hours.

**Exclusion criteria: Consider excluding those who currently, or have in the past 12 months, smoke/d cigarettes as they are known to influence your outcome measures, especially vascular function and blood pressure.**

Answer:

We absolutely agree with the reviewer on the influence that can have the tobacco on outcomes related to blood pressure and vascular function. In a first version of the study protocol, we had decided that smoking at the present time or having done it in the previous 12 months was a cause of exclusion. However, the potential difficulty in recruitment considering the selection criteria and the expected low frequency of women smokers in this age that can be around 10-12% (according to the clinical history data) finally changed our opinion. However, it is planned to include this condition as a control variable in the statistical analysis, so we have included this aspect in the section on methodological limitations and we have added a comment in the section on statistical analysis.

(Pag 15, line 397):

The smoking status in the 12 months prior to the time of inclusion could influence the outcome measures related to vascular function and blood pressure so, although participants will not be excluded for this reason, this aspect will be controlled in statistical analysis.

(Pag 14, line 383):



Effects of the intervention will be analyzed in a comparison of the changes in blood pressure and the secondary variables between the IG and the CG, using ANCOVA and adjusting for possible confounders as the smoking status. Effects of the intervention during follow-up will be studied with an analysis of the variance of repeated measures.

#### **Chocolate condition:**

- **Provide some justification for such a small amount of chocolate (10g per day) to be used.**

Answer:

The chocolate in the study has 99% cocoa, so its flavor is slightly more bitter and less sweet than the chocolate we are used to consuming, which could notably decrease the adherence to its consumption in the intervention group if the quantity indicated was greater. In the same way, the objective of this study is to assess the effect of adding a daily amount of chocolate to the habitual diet. In the EPIC-Norfolk cohort study [49] the median daily chocolate consumption was 4.6 g/day (IQR 0.6–12.0); among consumers only, median chocolate intake was 7.0 g/day (IQR 3.5–15.5). So we think that a daily amount of 10 g, which is almost double the average daily consumption in healthy adults may be enough to achieve the objectives. In addition, the EFSA (European Food Safety Authority) recommends an average consumption of 10 g of high- flavanol dark chocolate in the context of a balanced diet to achieve beneficial physiological effects on the vascular endothelium [50]. The daily contribution of energy is 59 kilocalories, so a greater daily amount of chocolate would also mean a greater contribution of energy, which could cause unwanted effects on body weight.

We have added a brief clarification on the choice of this amount of chocolate in the methodology section (Pag 8, line 205):

IG participants will be given chocolate with 99% cocoa content and asked to eat 10 g daily for a period of 6 months. According to the EFSA (European Food Safety Authority), 10 g of high-flavanol dark chocolate consumed in the context of a balanced diet could help maintain endothelium-dependent vasodilation [50].

- **You stated the chocolate will be consumed at the same time each day along with other eating instructions. State any recommendations for participants on the food and liquids consumed around this time to maximise the absorption of the cocoa polyphenols.**

Answer:

There are few studies that analyze the possible relationship between food intake and the absorption of cocoa polyphenols and, in addition, the results obtained are inconclusive. In the study by Keogh et al. [1] the milk slightly accelerated the absorption of polyphenols, although it did not influence the average concentration reached. Likewise, van het Hof et al. [2] concluded that the addition of milk to green tea or black tea did not affect plasma catechin levels. Wang et al. [3] observed in a study, in which chocolate was consumed with 40 g of bread, that the plasma concentration of epicatechin was higher than the average value achieved in a previous study conducted by the same group in which the subjects did not

consume any additional food (bread) until 4 h after the chocolate intake. However, these authors conclude that the influence of the bread on the bioavailability of epicatechin from chocolate must be determined in future studies.

Here are some of the recommendations that are given to the participants:

- The chocolate in the studio has 99% cocoa, so its flavor is slightly more bitter and less sweet than the chocolate that is usually consumed. According to daily consumption, you will get used to the taste and sensations you experience.
- It is recommended to consume the chocolate every day at about the same time.
- To improve the tasting you can consume the chocolate in small pieces leaving them unmated in the mouth without chewing, but refrain to melt the chocolate with milk.

We have introduced some of these instructions in the text (methodology section) (Pag 8, line 208):

Participants will also be given instructions on eating and keeping the product, with the recommendations, for example, that the chocolate can be consumed in small pieces leaving them unmated in the mouth, without chewing them. In addition, a series of recommendations will be given remembering the organoleptic characteristics of the product, as well as the recommendations of trying to consume the product at the same time or refrain from ingesting it dissolved in milk.

#### Measurements:

- **State the laboratory environment standardisation for this blood pressure and vascular function assessments. I.e. in a stimulating-free environment that limits surrounding noise; rest period, lighting and temperature standardised if possible.**

Answer:

In effect, there is a standardized protocol prior to each visit. We have written a summary of this protocol in the methodology section (Pag 13, line 334):

The evaluation visits will be made in the morning between 8:00 and 10:00 a.m. Each participant will be informed prior to the visit to go fasting for at least 12 hours, having avoided the 24 hours prior to visiting the consumption of polyphenol-rich foods, including cocoa, chocolate, apples, and red wine as well as alcoholic drinks or the performance of programmed physical activity. All evaluation visits, including blood pressure measurements and evaluations of vascular function, will be carried out in a room with conditions of lighting and temperature standardized, recommending that patients attend the appointment with a prior rest of at least 8- 10 hours.

- **It would be recommended to have a familiarisation session for your cognitive tests prior to baseline measurements to ensure limiting a learning effect over the subsequent 3 and 6 month testing periods.**

Answer:

In effect, an explanation and familiarization session of the cognitive tests is carried out prior to baseline measurements to ensure limiting a learning effect over the subsequent testing periods.

We have added the following paragraph in the methodology section (Pag 10, line 255):

The instructions are presented visually at the start of the baseline measurement to ensure limiting a learning effect over the subsequent testing periods.

- **In regards to body composition, postmenopausal women are at an increased risk of weight gain, particularly adipose tissue gain, due to the reduction of endogenous estrogen associated with the menopause transition. Therefore, assessing fat mass and body fat percentage would be the primary outcomes for your body composition measurements. Although I am not fully across the board with the literature surrounding the Inbody 230 Monitor in assessing body composition, the literature that I have read suggest that although lean body mass is accurately measured, body fat mass and body fat percentage are considerably underestimated by this machine. Is this equipment more accurate in repeated measures changes, rather than absolute values for fat mass and percentage? Have you considered using another body composition method in conjunction with this method such as DXA, or even skin folds?**

Answer:

We agree with the reviewer in pointing out that in postmenopausal women the main outcome for the evaluation of body composition should be both fat mass and body fat percentage. We have introduced a clarification in the text (Pag 11, line 282):

Body composition will be measured with the Inbody 230 Monitor [51]. This analyzer provides data on fat mass and body fat percentage as principal outcomes and also skeletal muscle mass, total body water, fat-free mass, waist-hip ratio, basal metabolism, and a segmental analysis.

On the other hand, we have not found studies that analyze body composition data in repeated measures using the Inbody 230, so we do not know its accuracy in this regard. However, our objective is not to analyze the absolute values of these variables but the changes produced. Since it is not the main outcome variable, we have not considered using another method such as DXA in parallel. We have used this same device that are already being used in other studies [52].

**Article to include: Marsh, et al, 2017. Brachial and cerebrovascular functions are enhanced in postmenopausal women after ingestion of chocolate with a high concentration of cocoa. Journal of Nutrition.**

**doi: <https://doi.org/10.3945/jn.117.250225>**

Answer:

We have included the reference of this work with a comment in the introduction section (Pag 5, line 112):

**Cocoa polyphenols and cognitive performance:** There is evidence to suggest that chocolate rich in polyphenols may be beneficial for cognitive performance and state since it improves mental processing speed and attenuates the increase of mental fatigue among healthy young adults [26, 27]. An improvement in cognitive performance among older age groups after eating chocolate has also been observed [28] and especially in subjects with higher risk of cardiovascular disease [29]. Several studies also show that polyphenol-rich chocolate causes an improvement in executive function and categorical fluency [30], in working memory [31, 32], and a slowing of mental fatigue [33] and also that a higher frequency of chocolate consumption has been associated with better cognitive function [32]. Furthermore, a positive influence of cocoa polyphenols on physiological processes has been reported, with a neuroprotective effect [34] and improved cognitive performance [35]. In this regard, it has been suggested that the brain-derived neurotrophic factor (BDNF) may play a role in the cognitive enhancement induced by the flavonoids [36]. Favorable effects on cerebrovascular function have also been observed in postmenopausal women after consumption of chocolate with high concentration of cocoa [37].

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