Trials

# Postprandial effect of breakfast glycaemic index on vascular function, glycaemic control and cognitive performance (BGI study): Study protocol for a crossover clinical trial.

--Manuscript Draft--

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| **Manuscript Number:** | TRLS-D-16-00075R1 |
| **Full Title:** | Postprandial effect of breakfast glycaemic index on vascular function, glycaemic control and cognitive performance (BGI study): Study protocol for a crossover clinical trial. |
| **Article Type:** | Study protocol |
| **Funding Information:** | Regional Health Management of Castilla and León (GRS 1192/B/15) — PhD Jose I Recio-Rodriguez |
| **Abstract:** | Background: Postprandial glycaemic response affects cognitive and vascular function. The acute effect of breakfast glycaemic index on vascular parameters is not sufficiently known. In turn, the influence of breakfasts with different glycaemic index on cognitive performance has been mostly studied in children and adolescents with varying results. Therefore, the purpose of this study is to analyse the postprandial effect of high and low glycaemic index breakfasts on vascular function and cognitive performance and their relationship with postprandial glycaemic response in healthy young adults.<br>Methods/Design: This is a crossover clinical trial targeted adults (aged 20 40 years, free from cardiovascular disease) selected by consecutive sampling at urban primary care health clinics in Salamanca (Spain). Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ). The postprandial effect will be assessed at 60 and 120 minutes from each breakfast including blood sampling and cognitive performance evaluations. Measurements of arterial stiffness and central hemodynamic parameters will be taken at -10, 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes.<br>Discussion: The differences in breakfast glycaemic index could affect vascular parameters, cognitive performance and postprandial glycaemic response with important applications and implications for general population. This provides necessary information for the establishment of new strategies in terms of nutritional education and work performance improvement.<br>Trial registration: ClinicalTrials.gov Identifier NCT02616276. Registered 19/11/2015. |
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| **Response to Reviewers:** | Dear Editor of Trials:<br><br>Thank you for your help in reviewing this STUDY PROTOCOL for consideration of publication in Trials.<br><br>Following the suggestions of the reviewers, we enclose a new version of our manuscript entitled: “Postprandial effect of breakfast glycaemic index on vascular function, glycaemic control and cognitive performance (BGI study): Study protocol for a crossover clinical trial” TRLS-D-16-00075, together with replies to all the issues raised.<br><br>GENERAL COMMENTS:<br>As the reviewers suggest, the background and discussion sections have been restructured to clarify the specific novel aspects of our study, some of the methods to be employed have been detailed and the entire manuscript has been reviewed by an English language professional (English editing certificate attached as supplementary material). These changes are shown underlined in the current study protocol. We think that its understanding is better now. We therefore believe that its interest has increased considerably.<br><br>We look forward to hearing from you. If you have any additional request or need any information, please contact us.<br><br>Sincerely:<br>Natalia Sánchez Aguadero,<br>Primary Care Research Unit, The Alamedilla Health Centre<br>Av. Comuneros 27, 37003 Salamanca, Spain.<br>Tel: +34 670 810587; Fax +34 923 123644<br>E mail: natalia.san.ag@gmail.com<br><br>Reviewer #1: A very interesting paper investigating the role of GI at breakfast on cognitive function, vascular function and insulineamia.<br><br>Answer to reviewer:<br><br>First, thank you for your work in reviewing this study protocol. Your contributions and suggestions will improve the understanding of the text.<br><br>The manuscript itself is a trial design only. All elements use standard protocols but this paper has decided to combine them to research its intended study aims.<br>Overall the paper is of good quality, but a number of clarifications are required before publication. I have listed specific concerns below:<br><br>1. The introduction is very clunky and needs to be restructured. There are 3 distinctive sections that are discussed but they would benefit from being linked together more clearly. Currently they read as if 3 standalone studies are stuck together. I would recommend the authors provide a clearer rationale for taking all 3 measures together at the same time in one study in the introduction.<br>In addition, the authors have reported data from studies using children and adolescent participants (lines 107:118). It would be more beneficial to explore studies in adult populations.<br><br>1.1. The introduction is very clunky and needs to be restructured. There are 3 distinctive sections that are discussed but they would benefit from being linked together more clearly. Currently they read as if 3 standalone studies are stuck together. I would recommend the authors provide a clearer rationale for taking all 3 measures together at the same time in one study in the introduction. |

Answer to reviewer:

Following the suggestions of the reviewer, the background has been restructured and its three distinctive sections have been linked together more clearly (Line 68, Page 3):

The glycaemic index (GI) is a measure of the speed with which a carbohydrate is absorbed compared to a reference product (pure glucose) [1, 2]. Diets with a high GI increase the risk of diseases related to chronic lifestyles such as type 2 diabetes mellitus [3, 4]. A recent meta-analysis of fourteen prospective studies found that high GI diets are associated with an increased risk of cardiovascular disease (CVD) [5] while a reduction in dietary GI can favourably affect the incidence of coronary disease in women [6]. Low GI diets might reduce the risk of CVD because they decrease postprandial glycaemia with different metabolic effects including differences in insulin sensitivity, circulating lipid concentrations and vascular function [3].
Regarding this latter aspect, the currently accepted gold standard to assess arterial stiffness is carotid-femoral pulse wave velocity (PWV) [7], which has been related to increased morbidity and mortality in both patients with CVD and healthy individuals [8, 9]. Likewise, the augmentation index (AIx) is a measure of wave reflection and arterial stiffness that has been shown to be a predictor of both future cardiovascular events and all-cause mortality [10]. In this way, the Conduit Artery Function Evaluation (CAFE) study [8] was designed to examine the impact of two different blood pressure (BP) lowering-regimens on derived central aortic pressures. It showed a greater morbidity and mortality and a higher central blood pressure (CBP) and AIx in one of the treatment groups versus the other with no differences in peripheral BP.
In turn, the Lifestyles and Vascular Aging (EVIDENT) study [11] analysed the relationship between lifestyle and arterial aging in a sample of 1553 subjects who were free of cardiovascular disease. We concluded that low GI diets were associated with lower AIx values. In this regard, a reduction in central hemodynamic parameters, AIx and PWV at 60 minutes from food intake has been reported in healthy adults perhaps because of an increase in insulin and/or visceral vasodilatation [12]. Another possible explanation for these findings might be the postprandial hypotension that occurs after a meal due to decreased cortisol secretion and activation of parasympathetic system [13]. For these reasons, although the effects of various macronutrients on vascular function have been explored in a number of studies [14-17], Taylor et al. [12] underlined the importance of analysing the impact of different types of meals on parasympathetic activity, CBP and vascular function parameters.
Of particular interest is the carbohydrate (CHO) content of a meal, which changes postprandial glucose and insulin levels and results in varying AIx reductions in postmenopausal women [18]. Thus, breakfast would play a fundamental role because low and high GI meals eaten in the morning have resulted in better glycaemic control versus eating in the evening [19]. However, despite the fact that breakfast patterns are associated with the metabolic profiles [20], few authors have studied its effect on cardiovascular responses. Ahuja et al. [21] found that a light breakfast (1301 KJ energy) reduced AIx, CBP and BP, and increased heart rate (HR) in adults versus fasting (water). In contrast, a trial aimed to compare the dietary effects of a high GI with a low GI breakfast replacement in obese and overweight individuals reported no differences in BP between breakfasts together with beneficial changes in fasting glucose and insulin levels unaffected [22].
On the other hand, increasing evidence has shown that the postprandial glycaemic response also has a potential impact on cognitive function [23]. Because breakfast is often the first meal before a long working day, there is interest in examining the influence of breakfasts with different GI on cognitive outcomes in healthy young adults. Cognitive performance may be influenced by many factors including individual differences, socioeconomic status or nutritional and health status [24]. The effect that different GI breakfasts may have on cognitive performance has been studied in people with type 2 diabetes mellitus and obesity, but it is not clear that a specific GI breakfast could benefit cognitive processes in these participants [23, 25]. However, various studies conducted on children have explored the relationship between breakfasts consisting of different GI foods and cognitive functions with contrasting results [24, 26-29]. It appears that a low GI breakfast can benefit the immediate [28-30] and delayed [31] verbal memory as well as sustained attention [30] and verbal fluency [32]. A high GI breakfast may confer benefits for selective attention, processing speed and working memory [32]. In addition, consuming different GI carbohydrates at breakfast could

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| | modulate cognitive performance, but this effect requires more study [33].<br><br>1.2. In addition, the authors have reported data from studies using children and adolescent participants (lines 107:118). It would be more beneficial to explore studies in adult populations.<br><br>Answer to reviewer:<br><br>As far as we know, this is the first study that assesses the postprandial effect of different types of breakfasts on cognitive performance in healthy young adults. The studies developed to date have been performed on samples of children and adolescent participants, due to the importance that cognitive functions have on academic development. Knowing the cognitive processes involved in the different cognitive functions studied (verbal and working memories, executive functions, attention, etc.) and the results found in children and adolescents, we have hypothesized that revealing results about this relationship could be found if we studied it in healthy young adults. However, this relationship has been previously studied in samples of people with type 2 diabetes and obesity. For this reason, as you can be seen above, we have introduced a mention to these studies in the background section (Line 116, Page 5).<br>2. I have a number of concerns regarding the methodology which need to be clarified:<br><br>2.1. Can the authors provide a rationale for measuring insulineamia at only 10, 60+ and 120+ minutes? Previous research investigating glucose and insulin response would encourage regular measurements at -5, 0, 15, 30, 45, 60, 90 and 120 mins (Henry et al; FAO/WHO).<br><br>Answer to reviewer:<br><br>We are aware of additional blood samples would improve the interpretation of the glycaemic response to consumption of each breakfast. However, we have a limited funding and we consider the glycaemic response as an intermediate variable between vascular function and cognitive performance. Because of that, we have established these three time point's blood samples.<br><br>2.2. Can the authors guarantee that all measurements will be completed at the assigned time there appears to be several measures collected at once which might result in a measure being taken later or earlier predicted?<br><br>Answer to reviewer:<br><br>We have performed a pilot study and we can guarantee that all measurements will be completed at the assigned time.<br><br>2.3. Would the measures interfere with each other?<br><br>Answer to reviewer:<br><br>The pilot study previously mentioned was performed with the objective of preventing the interference between measurements collected at once. In this sense, cognitive performance evaluations will be exactly completed at indicated minutes, cannula blood samples are planned to be collected next and Mobil-O-Graph® measurements will be scheduled to be performed automatically after.<br><br>2.4. Can the authors provide a rationale for the choice of breakfast foods?<br><br>Answer to reviewer:<br><br>The main objective of this study is to determine the effect of high GI breakfast versus low GI in the variables studied. That has been the reason for the choice of these products and not others.<br><br>2.5. Can the authors provide a rationale for providing chocolate as part of the breakfast? Are there more 'traditional' breakfasts that would be more suitable? Or is this representative of a typical breakfast in their area? |

Answer to reviewer:

Most of these products are consumed typically in the breakfast while others are not. The reason for providing chocolate as part of breakfast is its low GI. However, as indicated by the reviewer, a possible and interesting work could be to evaluate the effect of typical breakfast of a region or area as may be the Mediterranean breakfast. But we could discuss it in a future study.

2.6. More specifically can they confirm the GI values of the breakfasts and provide a comprehensive nutritional breakdown of the foods (energy, carbohydrate etc)

Answer to reviewer:

We have included the information about the macronutrient content and glycaemic index of these breakfasts in the methods section (Line 208-220, Page 8):

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

Additionally, we have introduced the information about the energy and glycaemic index in the abstract (methods) (Line 47, Page 2):

Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ).

2.7. It is suggested that the authors ensure participants limit their physical activity; alcohol and smoking at least 24 hours before each test. There is substantial evidence to suggest that these components alter glucose and insulin measurements up to 24 or 48 hours.

Answer to reviewer:

Taking into account the suggestions of the reviewer, the participants will be asked to limit their physical activity, alcohol consumption and smoking during 24-48 hours before each intervention visit.

We have included this information in the methods section (Line 201, Page 8):

Each of the three scheduled visits will last 2 hours 40 minutes and it will occur between 8:15 am and 10:55 am. Participants will be asked to fast for 12 hours overnight prior and to limit their physical activity, alcohol consumption and smoking during the previous 24-48 hours.

2.8. When and how long after breakfast will the Trail Making Test be completed?

Answer to reviewer:

The length of the cognitive performance evaluation will be 10 minutes and Trail Making Test will be scheduled at minute 5. Therefore, it will be completed 5 minutes before breakfast and 55 and 115 minutes after this.

Statistical analysis – The authors indicate that an independent t-test will be conducted to determine differences between breakfasts, however there are three arms (control, High GI and Low GI) and therefore surely a ANOVA is more suitable? This could then be stratified by gender, age, SES etc. as indicated (line 274)

Answer to reviewer:

We have changed the statistical analysis section, including some new paragraphs and reorganizing others, and now it reads as follows (Line 304, Page 11):

The normal distribution of variables will be verified using a Kolmogorov-Smirnov test. Quantitative variables will be displayed as the mean ± standard deviation if normally distributed or as the median (interquartile range) if asymmetrically distributed. The qualitative variables will be expressed as frequencies. The data will be quantitated using the Q of the Cochrane test for qualitative data as well as repeated measures for ANOVA or the Friedman test if the data is non-normally distributed for quantitative data. To compare the differences among the three types of breakfast in quantitative variables, ANOVA test will be used, or Wilcoxon test if the data is non-normally distributed. We will use Least Significant Difference (LSD) test as post hoc analysis and the Chi Square test in qualitative variables. The relationship of quantitative variables to each other will be tested using Pearson's or Spearman's correlation as appropriate. The effect of the interventions can be modified by age, gender, cultural and socioeconomic level, body mass index, lifestyles and last menstruation date. To control the effect of such confounding factors on the study results and to evaluate adequately the effect of the interventions, a multivariate analysis will be performed using the General Lineal Model (GLM) over in basic or extended models. The contrasting hypothesis will establish an alpha risk of 0.05 because the limit of statistical significance. The data will be analysed using the IBM SPSS Statistics for Windows version 23.0 (Armonk, NY: IBM Corp).

Reviewer #2: General comments to the Authors: The manuscript describes a study that would be of relevant to readers interested in clinical nutrition. In particular, the breakfast meal is topic generating significant interest of late and therefore this study would add to this developing body of literature. I do however think that the specific novel aspects of this study needs to be clarified more explicitly in the introduction and discussion sections of this manuscript. In addition, some of the methods employed are not explained in sufficient detail. In particular, the protocol used for measurement of blood pressure and the protocol used for collection, treatment, storage and analysis of blood samples.
There are also numerous examples in the manuscript of English language errors. I have highlighted some of these below, but I would advise the authors to seek assistance from an English language professional to improve the readability of the manuscript.
I have provided specific comments on each section of the manuscript below, along with a couple of notes about the protocol, which I hope the authors find useful.

Answer to reviewer:

First, thank you for your work in reviewing this study protocol. Your contributions and suggestions will improve the understanding of the text.

Specific comments to the Authors:

1. Title

1.1. As glucose and insulin is being assessed, consider changing 'insulinemia' to 'glycaemic control', as this incorporates the glucose and insulin components of the study.

Answer to reviewer:

As the reviewer suggests, we have changed “insulinemia” to “glycaemic control” in the title (Line 2, Page 1):

Postprandial effect of breakfast glycaemic index on vascular function, glycaemic control and cognitive performance (BGI study): Study protocol for a crossover clinical trial.

2. Abstract

2.1. Lines 30-36: The background section needs to articulate more explicitly the novel aspect of the study. It is clear that there are several aspects to this study, but why they are being investigated concurrently is not clear.

Answer to reviewer:

Following the reviewer’s suggestions, this section has been reworded to articulate more explicitly the novel aspect of the study (Line 38, Page 2):

Postprandial glycaemic response affects cognitive and vascular function. The acute effect of breakfast glycaemic index on vascular parameters is not sufficiently known. In turn, the influence of breakfasts with different glycaemic index on cognitive performance has been mostly studied in children and adolescents with varying results. Therefore, the purpose of this study is to analyse the postprandial effect of high and low glycaemic index breakfasts on vascular function and cognitive performance and their relationship with postprandial glycaemic response in healthy young adults.

2.2. Line 41: I think 'black chocolate' is generally referred to as 'dark chocolate'.

Answer to reviewer:

We have replaced the term “black chocolate” for “dark chocolate” in the abstract and the methods:

(Line 47, Page 2): Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ).

(Line 215, Page 8): This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

2.3. Lines 46-47: I don't think the '+' is required here as it is understood that times greater than 0 will automatically be positive. The '10' should remain though.

Answer to reviewer:

Taking into account the suggestion of the reviewer, we have deleted the “+” on the indicated line (Line 54, Page 2):

Measurements of arterial stiffness and central hemodynamic parameters will be taken at -10, 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes.

3. Background

3.1. This section requires some alteration as it is not made clear what the novel aspects of this study are. In addition, there are several examples of grammar mistakes and poor English (more than is mentioned below) that need to be corrected.

Answer to reviewer:

This section has been restructured to clarify the specific novel aspects of the current study. In addition, an English language professional has reviewed the entire manuscript. Thus, we attach the English editing certificate as supplementary material.

3.1.1. Line 77: I think more information is required than just 'CAFÉ sub-study' as this does not describe what was done.

Answer to reviewer:

The information required by the reviewer has been included in the text (Line 82, Page 3):

In this way, the Conduit Artery Function Evaluation (CAFE) study [8] was designed to examine the impact of two different blood pressure (BP) lowering-regimens on derived central aortic pressures. It showed a greater morbidity and mortality and a higher central blood pressure (CBP) and AIx in one of the treatment groups versus the other with no differences in peripheral BP.

3.1.2. Line 80: Reword to 'particularly 60 min after ingestion' or similar.

Answer to reviewer:

Following the reviewer's suggestion, we have modified this phrasing and now it reads as follows (Line 90, Page 4):

In this regard, a reduction in central hemodynamic parameters, AIx and PWV at 60 minutes from food intake has been reported in healthy adults perhaps because of an increase in insulin and/or visceral vasodilatation [12].

3.1.3. Line 80-82: This is unclear and needs rewording.

Answer to reviewer:

These statements come from the cohort study by Zanasi et al. [Zanasi A et al: J Hypertens 2012, 30(11):2125-2132], which assesses the prevalence and the prognostic power of postprandial hypotension in ambulatory hypertensive elderly patients without overt heart diseases. However, the background has been restructured to clarify the specific novel aspects of the current study, and we have thought that these sentences could be removed.

Thus, now the paragraph that included this information reads as follows (Line 92, Page 4):

Another possible explanation for these findings might be the postprandial hypotension that occurs after a meal due to decreased cortisol secretion and activation of parasympathetic system [13].

3.1.4. Line 82: A decrease in blood pressure is the same as hypotension, so for consistency just use one or the other.

Answer to reviewer:

As you can be seen above, now we only use the term "hypotension".

3.1.5. Line 85: The reference cited here was a short (4 h) intervention study investigating postprandial effects of MUFA and SFA meals. I fail to see how this study could determine that BP variation after breakfast is an independent risk factor for mortality.

Answer to reviewer:

This statement also comes from the cohort study by Zanasi et al. [Zanasi A et al: J Hypertens 2012, 30(11):2125-2132]. However, the background has been restructured to clarify the specific novel aspects of the current study, and we have thought that this sentence could be removed.

3.1.6. Lines 93-95: Needs rewording

Answer to reviewer:

We have reworded these statements (Line 99, Page 4):

Of particular interest is the carbohydrate (CHO) content of a meal, which changes postprandial glucose and insulin levels and results in varying AIx reductions in postmenopausal women [18].

3.1.7. Line 94: What is meant by 'different reductions'? Please clarify this.

Answer to reviewer:

“Different reductions” means that varying AIx reductions have been obtained. This has been clarified in the text (Line 99, Page 4):

Of particular interest is the carbohydrate (CHO) content of a meal, which changes postprandial glucose and insulin levels and results in varying AIx reductions in postmenopausal women [18].

3.1.8. Line 98: Please explain what is meant by the 'variability of CHO content' in breakfast. Generally, traditional breakfast foods tend to be high carbohydrate, so please explain this statement.

Answer to reviewer:

As noted by the reviewer, breakfast usually has an important load of carbohydrates. However, there can be variability in the glycaemic index of these carbohydrates. This was the idea that we wanted to express.

Nevertheless, as the background has been reworded to articulate more explicitly the novel aspect of the study, we have considered that this statement could be removed, and now the following sentence appears in its place (Line 101, Page 4):

Thus, breakfast would play a fundamental role because low and high GI meals eaten in the morning have resulted in better glycaemic control versus eating in the evening [19].

3.1.9. Lines 98-104: Several grammar and punctuation errors are present in this paragraph. Please review this.

Answer to reviewer:

All the errors highlighted have been reviewed. Moreover, we have sought assistance from an English language professional to improve the readability of the manuscript. Thus, we attach the English editing certificate as supplementary material.

3.1.10. Line 101: HOMA should be defined, but this simply refers to homeostatic model assessment, so perhaps HOMAIR is more accurate. In addition, this is a fasting measure of insulin resistance, so I'm unsure how this can be affected by breakfast. If this is meant as a chronic effect of consuming a low GI breakfast, this needs to be made clear.

Answer to reviewer:

The main objective of our study is to determine the acute effect of high versus low GI breakfasts in the variables studied, so it is out of context to consider HOMA in the text. For that reason, we have reworded the paragraph referred to this index (Line 17, Page 4):

In contrast, a trial aimed to compare the dietary effects of a high GI with a low GI breakfast replacement in obese and overweight individuals reported no differences in BP between breakfasts together with beneficial changes in fasting glucose and insulin levels unaffected [22].

3.1.11. Line 102: 'proven' is strong wording here. Consider revising.

Answer to reviewer:

Following the suggestions of the reviewer, this line has been reworded (Line 105, Page 4):

Ahuja et al. [21] found that a light breakfast (1301 KJ energy) reduced AIx, CBP and BP, and increased heart rate (HR) in adults versus fasting (water).

3.1.12. Line 102: What is meant by a 'light' breakfast? Please provide energy content to clarify this.

Answer to reviewer:

We have included in this sentence the energy content of the breakfast, and now it reads as follows (Line 105, Page 4):

Ahuja et al. [21] found that a light breakfast (1301 KJ energy) reduced AIx, CBP and BP, and increased heart rate (HR) in adults versus fasting (water).

3.1.13. Lines 105-106: Please revise this sentence.

Answer to reviewer:

This sentence has been revised and its wording modified (Line 111, Page 5):

On the other hand, increasing evidence has shown that the postprandial glycaemic response also has a potential impact on cognitive function [23]. Because breakfast is often the first meal before a long working day, there is interest in examining the influence of breakfasts with different GI on cognitive outcomes in healthy young adults.

3.1.14. Line 114: consider changing to 'reporting contrasting results'

Answer to reviewer:

Following the suggestions of the reviewer, we have changed this sentence (Line 119, Page 5):

However, various studies conducted on children have explored the relationship between breakfasts consisting of different GI foods and cognitive functions with contrasting results [24, 26-29].
3.1.15. Line 114-118: Please revise this section. I'm not sure that significant is required in either of these sentences.

Answer to reviewer:

As the reviewer suggests, this section has been revised and its wording modified (Line 122, Page 5):

It appears that a low GI breakfast can benefit the immediate [28-30] and delayed [31] verbal memory as well as sustained attention [30] and verbal fluency [32]. A high GI breakfast may confer benefits for selective attention, processing speed and working memory [32].

3.1.16. Line 125: Remove 'and last'.

Answer to reviewer:

We have removed “and last” in this sentence (Line 133, Page 5):

The third goal is to analyse the association between postprandial glycaemic response and vascular function and cognitive performance for high versus low glycaemic index breakfasts.

4. Methods

4.1. More information is required on some of the measures to be taken. These are detailed below. Again, several grammatical errors are present in this section which requires revision.

Answer to reviewer:

The information required by the reviewer on some of the measures to be taken has been detailed below. In addition, an English language professional has reviewed the entire manuscript. Thus, we attach the English editing certificate as supplementary material.

4.1.1. Line 186: Change 'women' to female.

Answer to reviewer:

We have changed “women” to “female” and now this subheading reads as follows (Line 249, Page 9):

Female-specific variables.

4.1.2. Lines 187-189: Ideally stage of menstrual cycle should be controlled. This can be done by having ~1 month between trials (dependant on duration of menstrual cycle) and testing subjects between ~5-12 days after start of menstruation. Alternatively, only recruiting female subject on a monophasic contraceptive pill, and ensuring testing occurs in the active 21 day period.

Answer to reviewer:

We agree with the reviewer’s assessment that the stage of menstrual cycle should ideally be controlled. However, we have established a washout period of one week between each intervention for all the participants. Thus, the last menstruation date will be used as control variable on the study results for evaluating adequately the effect of the interventions.

4.1.3. Lines 197-200 Is waist and hip circumference measured in duplicate? This should be stated as the variation of circumference is likely to be larger than weight, which is measured in duplicate, as stated on line 194.

Answer to reviewer:

Waist and hip circumference will be measured in duplicate, following the recommendations of the Spanish Society for the Study of Obesity (SEEDO) [Salas-Salvado J et al: Med Clin (Barc) 2007, 128:184-196; quiz 181 p following 200].

We have stated this in the text (Line 260, Pag 10):

Following the recommendations of the Spanish Society for the Study of Obesity (SEEDO) [36], the waist circumference will be measured in duplicate at level of the midpoint between the last rib and the iliac crest with the subject standing without clothing using a flexible tape parallel to the floor after inspiration. Hip circumference will be similarly measured at the level of the trochanters.

4.1.4. Lines 201-211: What procedures will be followed before measures of blood pressure are taken? Will subjects be seated, standing, supine etc? Will there be a period of rest before measurement? This information need to be included here.

Answer to reviewer:

Following the reviewer's suggestions, we have added a new paragraph, which includes this information (Line 268, Page 10):

They will be made on the participants' dominant arm in a seated position after at least 5 minutes of rest with a cuff of appropriate size as determined by measurement of the upper-arm circumference and following the recommendations of the European Society of Hypertension [37].

4.1.5 Line 211: As above, remove the '+' here.

Answer to reviewer:

As the reviewer suggests, we have removed the "+" on the indicated line (Line 277, Page 10):

This device will be scheduled to perform continuous measurements at -10, 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes with the subject sitting and resting his arm on a rigid surface.

4.1.6. Lines 212-217: How are blood samples collected, treated, centrifuged and stored? How will blood samples be analysed?

Answer to reviewer:

As the information provided in this section was insufficient, we have detailed all the issues raised by the reviewer in the text (Line 281, Page 11):
At the time of study entry and prior to the first intervention visit, fasting plasma creatinine, serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides values will be determined using standard enzymatic automated methods.
During each study visit, three cannula blood samples will be collected at -10, 60 and 120 minutes to measure serum glucose and insulin levels by ultraviolet-visible spectrophotometry and chemiluminescence, respectively. Serum will be isolated by centrifugation and stored at a – 20 ºC freezer within 48-72 hours until analysis.
Samples will be treated and centrifuged by a single researcher under standardized conditions. The analysis will be performed in a laboratory in external quality assurance programs of the Spanish Society of Clinical Chemistry and Molecular Pathology.

4.1.7. Lines 228-258: Remove 'it' and 'minute' from the beginning of sentences in this section. As above, remove the '+' here as well.

Answer to reviewer:

Following the suggestions of the English language professional sought, we have replaced "it" for "this" in these sentences (Lines 206-220, Page 8):

Control breakfast (BF-1):
This will consist of 350 mL of water served at room temperature.

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index

of 29.4.

As the point 4.1.11.1 shows, the “Structure of intervention visits” section has been reworded and the other changes suggested by the reviewer have not been required.

4.1.8. Line 231: water served at room temperature.

Answer to reviewer:

We have introduced this modification in the “nutritional composition of each intervention arm” section (Lines 206-220, Page 8):

Control breakfast (BF-1):
This will consist of 350 mL of water served at room temperature.

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

4.1.9. Line 231: Why was 350 mL of water provided for the control condition? How was this calculated? The foods provided for each breakfast condition will contain water, and ideally this should be balanced between all 3 conditions.

Answer to reviewer:

The study by Ahuja et al [Ahuja KD et al: Am J Clin Nutr 2009, 90(2):298-303] provides abundant evidence of the effects of food intake on postprandial blood pressure and measures of arterial stiffness. In this work, “Participants were then provided with either 350 mL water (room temperature)”. The main results indicate that water intake did not elicit a significant change in brachial or central systolic pressure and the other variables, including aortic pressure and the augmentation index. The experimental group, besides water, took a light meal of 1301 KJ.
For these reasons, we decided to keep as a control group the intake of 350 mL of water served at room temperature, also including it in the rest of breakfasts, since their inclusion would not change the GI.

4.1.10. Lines 232-240: Please provide the macronutrient content of these breakfasts. Remove the decimal place from the energy content of the breakfast foods. Consider reporting energy in kJ alongside/instead of kcal, as this is the SI unit for energy. Could the overall GI value of each breakfast also be stated? Could you also explain the reason for food choices? Are these food items that would typically be consumed at breakfast? Providing typical breakfast foods would improve the ecological validity of the study.

4.1.10.1. Lines 232-240: Please provide the macronutrient content of these breakfasts. Remove the decimal place from the energy content of the breakfast foods. Consider reporting energy in kJ alongside/instead of kcal, as this is the SI unit for energy. Could the overall GI value of each breakfast also be stated?

Answer to reviewer:

We have included the information about the macronutrient content and glycaemic index of these breakfasts in the methods section (Line 208-220, Page 8):

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

Additionally, we have introduced the information about the energy and glycaemic index in the abstract (methods) (Line 47, Page 2):

Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ).

4.1.10.2. Could you also explain the reason for food choices? Are these food items that would typically be consumed at breakfast? Providing typical breakfast foods would improve the ecological validity of the study.

Most of these products are consumed typically in the breakfast while others are not. However, the main objective of this study is to determine the effect of high GI breakfast vs. low GI in the variables studied. That has been the reason for the choice of these products and not others. However, as indicated by the reviewer, a possible and interesting work could be to evaluate the effect of typical breakfast of a region or area as may be the Mediterranean breakfast. But we could discuss it in a future study.

4.1.11. Lines 241-258: What is a 'laboratory test sample'? If it is a blood sample, please state as such. The rationale for the time point's blood samples are being collected is sound, but additional blood samples, particularly between 0-60 min, would improve the interpretation of the glycaemic response to consumption of each breakfast.

4.1.11.1. Lines 241-258: What is a 'laboratory test sample'? If it is a blood sample, please state as such.

Answer to reviewer:

In order to improve the understanding of this section, we have reworded it and now appears out of the intervention section as “Study procedures”, where we have used the term “blood sample” following the reviewer’s suggestion (Line 187, Page 7):

On arrival at the research unit, subjects will be weighed, and their height, waist circumference and hip circumference will be measured. Participants will be seated and remain in this position throughout the visit. After 5 minutes of rest, a peripheral blood pressure measurement will be performed. Immediately, the central blood pressure and hemodynamic parameters will be obtained. Next, cognitive performance will be assessed and fasting blood samples will be collected and central blood pressure and hemodynamic parameters will be determined again. Subjects will be provided with a randomly assigned breakfast to be consumed within 10 minutes. At the first bite, a timer will be started and additional measurements of central blood pressure and hemodynamic parameters will be taken every 15 minutes. Furthermore, another two cognitive performance evaluations and postprandial blood sampling will be completed at 60 and 120 minutes.

4.1.11.2. The rationale for the time point's blood samples are being collected is sound, but additional blood samples, particularly between 0-60 min, would improve the interpretation of the glycaemic response to consumption of each breakfast.

Answer to reviewer:

We are aware of additional blood samples would improve the interpretation of the glycaemic response to consumption of each breakfast. However, we have a limited funding and we consider the glycaemic response as an intermediate variable between vascular function and cognitive performance. Because of that, we have established these three time point's blood samples.

5. Statistical analysis

5.1. It is stated that qualitative and quantitative data will be collected, but I am unsure which variables are qualitative. It might be useful to state the qualitative and quantitative variables, and how these will be handled statistically, separately.

Answer to reviewer:

The main results will be measured from quantitative variables (i.e. central and peripheral blood pressure, augmentation index, central hemodynamic parameters, laboratory and cognitive performance variables, etc.) that will be analysed with the ANOVA test or its corresponding non-parametric test, correlation and regression. However, we will also use the corresponding tests for the analysis of the relationship between certain qualitative variables, such as gender, smoking habits and some clinical and demographic data. Besides this, some of the quantitative variables will be categorized, according to previously established cut points.

5.2. It is stated on line 265 how data will be reported if normal or non-normally distributed, but how the data is handled if non-normally distributed is not stated in the statistical analysis section.

Answer to reviewer:

We have changed the statistical analysis section, including some new paragraphs and reorganizing others, and now it reads as follows (Line 304, Page 11):

The normal distribution of variables will be verified using a Kolmogorov-Smirnov test. Quantitative variables will be displayed as the mean ± standard deviation if normally distributed or as the median (interquartile range) if asymmetrically distributed. The qualitative variables will be expressed as frequencies. The data will be quantitated using the Q of the Cochrane test for qualitative data as well as repeated measures for ANOVA or the Friedman test if the data is non-normally distributed for quantitative data. To compare the differences among the three types of breakfast in quantitative variables, ANOVA test will be used, or Wilcoxon test if the data is non-normally distributed. We will use Least Significant Difference (LSD) test as post hoc analysis and the Chi Square test in qualitative variables. The relationship of quantitative variables to each other will be tested using Pearson's or Spearman's correlation as appropriate. The effect of the interventions can be modified by age, gender, cultural and socioeconomic level, body mass index, lifestyles and last menstruation date. To control the effect of such confounding factors on the study results and to evaluate adequately the effect of the interventions, a multivariate analysis will be performed using the General Lineal Model (GLM) over in basic or extended models. The contrasting hypothesis will establish an alpha risk of 0.05 because the limit of statistical significance. The data will be analysed using the IBM SPSS Statistics for Windows version 23.0 (Armonk, NY: IBM Corp).

5.3. Main effects should also be assessed before post-hoc analyses (i.e. t-tests) are performed. In this case, repeated measures ANOVA may be an appropriate way of handling the quantitative data.

Answer to reviewer:

We have modified the wording of the section referred to the main effects assessment, and now it reads as follows (Line 307, Page 12):

The data will be quantitated using the Q of the Cochrane test for qualitative data as well as repeated measures for ANOVA or the Friedman test if the data is non-normally distributed for quantitative data. To compare the differences among the three types of breakfast in quantitative variables, ANOVA test will be used, or Wilcoxon test if the data is non-normally distributed. We will use Least Significant Difference (LSD) test as post hoc analysis and the Chi Square test in qualitative variables.

6. Discussion

6.1. Line 307: What is the EVIDENT study? I would urge you to avoid stating abbreviated study names in general.

Answer to reviewer:

Following the suggestions of the third reviewer, this piece of evidence has been moved to the background section (Line 87, Page 4). However, we have included more information about the EVIDENT study:

In turn, the Lifestyles and Vascular Aging (EVIDENT) study [11] analysed the relationship between lifestyle and arterial aging in a sample of 1553 subjects who were free of cardiovascular disease. We concluded that low GI diets were associated with lower AIx values.

6.2. Line 314-315: This appears to be the central aims of the study, but the response of insulin to breakfasts of different glycaemic indexes is not particularly novel. The rationale for this part of the study requires more detail.

Answer to reviewer:
Our objective is really to analyse the relationship of vascular and cognitive function with postprandial glycaemic response for high versus low glycaemic index breakfasts. For this reason, we have modified the wording of the results expected (Line 339, Page 13):

In this project, we expect to demonstrate that low glycaemic index breakfast has a favourable effect on measures of vascular function and glycaemic response relative to high glycaemic index.

6.3. Line 316: Is 'similarly' correct here? I'm not sure these two aims are similar. Consider changing to 'secondly'.

Answer to reviewer:

Following the reviewer’s suggestions, we have changed “similarly” to “secondly” (Line 342, Page 13):

Secondly, based on the findings of previous studies conducted on children and adolescents [26-30, 32], we hypothesize that we will see similar effects in healthy young adults.

6.4. Lines 322-323: In this acute setting, I think the word 'improves' should be considered carefully. The acute effects of a given intervention are unlikely to improve plasma insulin values, for example. Therefore, I would suggest being more specific with this sentence, either explaining specifically the anticipated response to a given glycaemic breakfast, or perhaps use 'produces a favourable insulin response' in place of 'improve'.

Answer to reviewer:

The indicated lines have been reworded for improving their clarity (Line 348, Page 13):

Therefore, according to our hypothesis, the results of the current study may explain the influence of glycaemic index on cognitive and vascular function. Moreover, given the

high worldwide prevalence of cardiovascular diseases and its close relationship with cognitive decline, it would be interesting to know how the vascular parameters and cognitive processes that are affected by the type of breakfast consumed and as a function of lifestyle and dietary interventions.

7. Table 1

7.1. I'm not sure what this table is supposed to show, and what the 'X' in the box means. If this simply states what will be done at each visit, I'm not sure it adds anything over what is stated in the text. What is the 'basal evaluation'? Basal is a specific term, meaning minimum requirement for sustaining life. For example, BMR is the amount of energy required to maintain basic celular processes to sustain life exclusively, and is not the same as RMR, which includes a 'resting' component to account for nonessential (albeit minimal) energy requirement processes. Therefore, I'm unsure what basal means in the context of this table.

Answer to reviewer:

This table shows what will be done at baseline evaluation (which erroneously appears as basal evaluation) and at each intervention visit. As noted by the reviewer, it is not particularly informative, so we think that it can be removed.

8. Figure

8.1. Replace 'o' for 'or', as stated at each visit.

Answer to reviewer:

We have corrected this typographical error and we have replaced “basal evaluation” for “baseline evaluation”.

Reviewer #3 Dr Enhad Chowdhury: Sanchez-Aguadero present a protocol paper describing the rationale and methods for a clinical trial examining the effect of breakfast GI upon a range of outcomes. While this study has the potential to contribute evidence in several areas, the description of, and rationale behind the study, requires greater clarity throughout.

Answer to reviewer:

First, thank you for your work in reviewing this study protocol. Your contributions and suggestions will improve the understanding of the text.

1. General comments:

1.1. Throughout the manuscript, the authors refer to the particular importance of breakfast due to its variable carbohydrate content-is there any evidence to suggest that the carbohydrate content of the breakfast meal is more variable than other eating occasions within populations? If not, I do not think that breakfast should be highlighted in this way, as any meal can be manipulated to the same extent for GI.

Answer to reviewer:

As breakfast usually has an important load of carbohydrates, there can be variability in the glycaemic index of these carbohydrates. This was the idea that we wanted to express.

Nevertheless, as the background has been reworded to articulate more explicitly the novel aspect of the study, we have considered that this statement could be removed, and now the following sentence appears in its place (Line 101, Page 4):

Thus, breakfast would play a fundamental role because low and high GI meals eaten in the morning have resulted in better glycaemic control versus eating in the evening [19].

1.2. At several points in the manuscript the authors refer to evidence "proving" certain

effects. I would advise this phrasing could be removed in all cases and is particularly inappropriate when comparing opposing results from different studies.

Answer to reviewer:

The phrasing mentioned has been removed in all cases.

2. Specific comments

2.1. Abstract

2.1.1. Lines 32-34, the authors state "on the other hand, in terms of the influence of breakfasts with different glycaemic index on cognitive performance, different results have been obtained". It needs to be clarified whether the authors mean varying results have been obtained between studies (which I believe is the meaning) or that these results are different to others already stated. It needs to be clearer what the "different" refers to.

Answer to reviewer:

“Different” refers to varying results obtained between studies. This has been clarified in the abstract (Line 38, Page 2):

Postprandial glycaemic response affects cognitive and vascular function. The acute effect of breakfast glycaemic index on vascular parameters is not sufficiently known. In turn, the influence of breakfasts with different glycaemic index on cognitive performance has been mostly studied in children and adolescents with varying results. Therefore, the purpose of this study is to analyse the postprandial effect of high and low glycaemic index breakfasts on vascular function and cognitive performance and their relationship with postprandial glycaemic response in healthy young adults.

2.1.2. 40-42, if space allows then greater detail relating to the breakfast would be useful here, such as GI or energy content of breakfasts.

Answer to reviewer:

Following the suggestions of the reviewer, we have introduced the information about the energy and glycaemic index of each breakfast in the abstract (methods) (Line 47, Page 2):

Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ).

In addition, we have included the macronutrient content, energy and glycaemic index of each breakfast on the methods section, as the point 2.7.1 shows.

2.1.3. Lines 52- authors state improvement in "school and work performance". As this study is in adults and there is extensive literature regarding the effect of breakfast consumption in children the reference to school should be removed here.

Answer to reviewer:

Following the suggestions of the reviewer, the current wording of the discussion section in the abstract does not include the reference to school (Line 57, Page 3):

The differences in breakfast glycaemic index could affect vascular parameters, cognitive performance and postprandial glycaemic response with important applications and implications for general population. This provides necessary information for the establishment of new strategies in terms of nutritional education and work performance improvement.

2.2. Background

2.2.1 Lines 63-68, the authors make several points relating to the distinction between GI and GL but this study only seeks to manipulate GI-is there a specific reason both need contrasting here?

Answer to reviewer:

As the reviewer suggests, this study only seeks to manipulate GI and the distinction between GI and GL in the text is out of the context.

We have modified the text and now it reads as follows (Line 69, Page 3):

Diets with a high GI increase the risk of diseases related to chronic lifestyles such as type 2 diabetes mellitus [3, 4]. A recent meta-analysis of fourteen prospective studies found that high GI diets are associated with an increased risk of cardiovascular disease (CVD) [5] while a reduction in dietary GI can favourably affect the incidence of coronary disease in women [6].

2.2.2. Lines 74-76, the authors finish these lines refer to their chosen measures with the statement "complement the information obtained with PWV". Can it be clarified if their chosen measures are ordinarily used as an addition to this gold standard measure or are used as a surrogate measure for arterial stiffness on their own? If this is the case, a reference should be provided.

Answer to reviewer:

These parameters are measures of arterial stiffness on their own. This has been clarified and properly referenced in the text (Line 80, Page 3):

Likewise, the augmentation index (AIx) is a measure of wave reflection and arterial stiffness that has been shown to be a predictor of both future cardiovascular events and all-cause mortality [10].

2.2.3. Line 79-80, the authors state food intake "causes a reduction in central hemodynamic parameters, Aix and PWV in healthy adults, particularly after 60 minutes"-the study referred to only obtained postprandial measures at 60 and 180 minutes. Therefore, I would advise the "particularly" here is inappropriate unless another reference can be provided to suggest the effect is less in the first 60 minutes.

Answer to reviewer:

Following the reviewer’s advice, we have modified the wording of this statement and now it reads as follows (Line 90, Page 4):

In this regard, a reduction in central hemodynamic parameters, AIx and PWV at 60 minutes from food intake has been reported in healthy adults perhaps because of an increase in insulin and/or visceral vasodilatation [12].

2.2.4. Lines 80-84, there are several statements here where it is not clear where this information comes from e.g. "postprandial hypotension occurring in more than 70% of patients", "patients with higher BP values are those who experience it most". These statements should be clearly referenced.

Answer to reviewer:

These statements come from the cohort study by Zanasi et al. [Zanasi A et al: J Hypertens 2012, 30(11):2125-2132], which assesses the prevalence and the prognostic power of postprandial hypotension in ambulatory hypertensive elderly patients without overt heart diseases. However, the background has been restructured to clarify the specific novel aspects of the current study, and we have thought that these sentences could be removed.
Thus, now the paragraph that included this information reads as follows (Line 92, Page

4):

Another possible explanation for these findings might be the postprandial hypotension that occurs after a meal due to decreased cortisol secretion and activation of parasympathetic system [13].

2.2.5. Lines 84-85, "This variation in BP, and in particular that occurring after breakfast, it is an independent predictor of mortality" The referenced study did not assess mortality.

Answer to reviewer:

This statement also comes from the cohort study by Zanasi et al. [Zanasi A et al: J Hypertens 2012, 30(11):2125-2132]. However, the background has been restructured to clarify the specific novel aspects of the current study, and we have thought that this sentence could be removed.

2.2.6. Lines 87-92, I am not sure the specific comparisons of differing fatty acid compositions is particularly relevant for the current study.

Answer to reviewer:

The specific comparisons of differing fatty acid compositions were included in the background in order to express that the impact of certain types of meals on vascular function has been tested.

As noted by the reviewer, this information is not particularly relevant for the current study, so we have removed it and now we only mention that a number of studies have been conducted for testing the effects of various macronutrients on vascular function (Line 95, Page 4):

For these reasons, although the effects of various macronutrients on vascular function have been explored in a number of studies [14-17], Taylor et al. [12] underlined the importance of analysing the impact of different types of meals on parasympathetic activity, CBP and vascular function parameters.

2.2.7. Lines 93-95 are not clear. The study cited provided a fixed meal but this section gives the impression that differing CHO intake resulted in differing reductions in outcomes in healthy adults and postmenopausal women the study did not make a comparison between these groups and refers to the participants as "young and healthy".

Answer to reviewer:

These findings had not been correctly referenced. They come from a study conducted by Greenfield et al. in postmenopausal women [Greenfield JR: Int J Cardiol. 2007 Jan 2;114(1):50-6].

We have introduced the correct reference in the text (Line 99, Page 4):

Of particular interest is the carbohydrate (CHO) content of a meal, which changes postprandial glucose and insulin levels and results in varying AIx reductions in postmenopausal women [18].

2.2.8. Lines 102-104, after providing some contrary evidence the authors state that "the most important trial" is by Ahuja et al but don't provide a rationale for this. This is particularly important as the study by Ahuja did not compare GI but simply food vs no food, unlike the study cited in opposition which compared GI.

Answer to reviewer:

We agree with the reviewer that the study by Ahuja et al should not be considered as the most important. For that reason, we have changed the wording of this paragraph (Line 103, Page 4):

However, despite the fact that breakfast patterns are associated with the metabolic profiles [20], few authors have studied its effect on cardiovascular responses. Ahuja et al. [21] found that a light breakfast (1301 KJ energy) reduced AIx, CBP and BP, and increased heart rate (HR) in adults versus fasting (water). In contrast, a trial aimed to compare the dietary effects of a high GI with a low GI breakfast replacement in obese and overweight individuals reported no differences in BP between breakfasts together with beneficial changes in fasting glucose and insulin levels unaffected [22].

2.3. Objectives

2.3.1. Lines 122-124, is there a specific reason that the postprandial effect upon glucose and insulin is an objective here. The differences between these measures for high vs low GI meals is already very well established. This has been measured comprehensively in other studies. If it is the relationship between these measures and arterial stiffness that is the interesting comparison then this should be stated more clearly.

Answer to reviewer:

As noted by the reviewer, with respect to the glycaemic control, our objective is really to analyse the relationship of vascular and cognitive function with postprandial glycaemic response for high versus low glycaemic index breakfasts.

Due to that, we have reworded the objectives in the abstract and methods, and now they read as follows:

(Line 42, Page 2): Therefore, the purpose of this study is to analyse the postprandial effect of high and low glycaemic index breakfasts on vascular function and cognitive performance and their relationship with postprandial glycaemic response in healthy young adults.

(Line 128, Page 5): The primary objective of this study is to evaluate the postprandial effect of low and high glycaemic index breakfasts on vascular function as measured by central blood pressure, augmentation index and pulse wave velocity in a sample of healthy young adults. The secondary aim is to assess the postprandial effect of low and high glycaemic index breakfasts on cognitive performance in a sample of healthy young adults. The third goal is to analyse the association between postprandial glycaemic response and vascular function and cognitive performance for high versus low glycaemic index breakfasts.

2.4. Methods/Design

2.4.1. Line 132, I would advise referring to this as a control/fasting trial (as only water is being consumed) rather than a control breakfast as this might lead the reader to think this will be a reference breakfast (i.e that this might be a "normal" breakfast rather than no breakfast).

Answer to reviewer:

The study by Ahuja et al [Ahuja KD et al: Am J Clin Nutr 2009, 90(2):298-303] provides abundant evidence of the effects of food intake on postprandial blood pressure and measures of arterial stiffness. In this work, “Participants were then provided with either 350 mL water (room temperature)”. The main results indicate that water intake did not elicit a significant change in brachial or central systolic pressure and the other variables, including aortic pressure and the augmentation index. The experimental group, besides water, took a light meal of 1301 KJ.
For these reasons, we decided to keep as a control group the intake of 350 mL of water served at room temperature.

2.4.2. Lines 144-145, needs rewording. It is also not clear by what is meant by "consecutive sampling will ensure comparability of the groups" as this is a crossover trial so there is only one group?

Answer to reviewer:

We have rewording this line, which now reads as follows (Line 154, Page 6):

Because this is a crossover clinical trial, a consecutive sampling will ensure the comparability of interventions.

2.4.3. Lines 154-155, is there a specific definition of what constitutes "excessive consumption of toxic substances" as an exclusion criteria?

Answer to reviewer:

Any consumption of toxic substances will be excluded, so we have modified the wording of this exclusion criterion (Line 164, Page 6):

Subjects will be excluded with a history of cardiovascular events (acute myocardial infarction, stroke, etc.), hypertension, diabetes mellitus, dyslipidaemia, pharmacological treatment for any of these conditions, neurological and/or neuropsychological disease, or the consumption of toxic substances.

2.5. Sample Size

2.5.1. Lines 160-162, "Considering the study design with three groups" is followed by "40 subjects would be required in each group to detect a minimum difference…..between two groups". Firstly there are no groups here but only several trials in one group and also the number of groups referred to changes. Are the authors confident they have conducted an appropriate calculation accounting for the repeated measures design-this needs clarification?

Answer to reviewer:

Considering the design of paired data within each type of breakfast, and comparing them to the control condition, the sample size has been recalculated, by Granmo software (https://www.imim.es/ofertadeserveis/es_granmo.html), and the corresponding section reworded, as you can be seen at point 2.5.2.

2.5.2. Line 162 "to detect a minimum difference of 7.5 points in Aix". What is the basis of this difference being relevant? Clinical relevance or based upon other research findings? Also a minimum difference of 7.5 points at a specific timepoint, or an incremental measure over the time course? This needs clarification.

Answer to reviewer:

The CAFE study [Williams B et al: Circulation 2006, 113(9):1213-1225] found a reduction in the composite outcome of total cardiovascular events in the amlodipine regimen group, compared to the atenolol regimen group. There was a similar peripheral blood pressure decrease between treatment groups, but greater reductions in central blood pressure (4.3 (3.3, 5.4) mmHg) and central augmentation index (6.5 (5.8, 7.3) units) with the amlodipine regimen.

We have included this information in the sample size paragraph, and now it reads as follows (Line 179, Page 7):

The primary outcome variable is change in central augmentation index (AIx). The CAFE study [8] found a reduction in cardiovascular events associated with a decline of 6.5 (5.8, 7.3) points in the Aix. This is the basis of our calculation. The power calculation was a repeated measures design and compared both intervention breakfasts with a control breakfast with an alpha risk of 0.05 and a beta risk of 0.2. The SD was 10 with a correlation coefficient between the initial and final measurement of 0.7. Thus, 40 subjects are required to detect a minimum difference of 5 points in the AIx between two intervention breakfasts. A loss to follow-up of 5% was estimated.

2.6. Variables and measurement instruments

2.6.1 Line 177, it is stated that regular diet will be assessed-is it to be standardised before trials (i.e the day before/morning of trials) as this may affect some of the metabolic parameters to be measured?

Answer to reviewer:

The regular diet, together with other lifestyles, is going to be used as control variable on the study results for evaluating adequately the effect of the interventions.

2.6.2. Lines 187-188, "The date of the last menstruation will be recorded due to the effect it may have on the study variables". Is this information going to be used in any way? Is date from menses going to first trial to be standardised between participants to attempt to control for any effects of time from menses.

Answer to reviewer:

Although the stage of menstrual cycle should ideally be controlled, we have established a washout period of one week between each intervention for all the participants. Thus, the last menstruation date will be used as control variable on the study results for evaluating adequately the effect of the interventions.

2.6.3. Line 208, papers using augmentation index e.g. ref 13 use an augmentation index normalised to HR, is this to be employed in this study? This should be clarified ideally with some rationale.

Answer to reviewer:
We are going to employ an augmentation index corrected to a HR of 75 bpm. This has been clarified in the text (Line 274, Page 10):

The Mobil-O-Graph® device [38] will be used to estimate cardiac output and total peripheral vascular resistance and to measure central blood pressure, pulse wave velocity, reflection coefficient and augmentation index. This is affected by heart rate (HR) so its values will be corrected to a HR of 75 bpm. This device will be scheduled to perform continuous measurements at -10, 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes with the subject sitting and resting his arm on a rigid surface.

2.6.4 Lines 213-215, there are numerous measures listed here to be obtained upon study entry but what is the purpose of obtaining these measures?

Answer to reviewer:

These laboratory measures are going to be used as indicators of the inclusion criteria compliance and as control variables on the study results for evaluating adequately the effect of the interventions.

2.6.5 Line 213-217, for variables to be measured from blood samples greater detail should be provided for collection and analysis methods-e.g. (venupuncture/fingerprick/cannula) as well as detailing analysis medium (whole blood/plasma/serum) and specific equipment and methods to be employed.

Answer to reviewer:

We agree with the reviewer’s assessment that the information provided in this section was insufficient, so we have detailed all the issues raised (Line 281, Page 11):

At the time of study entry and prior to the first intervention visit, fasting plasma creatinine, serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides values will be determined using standard enzymatic automated methods.
During each study visit, three cannula blood samples will be collected at -10, 60 and 120 minutes to measure serum glucose and insulin levels by ultraviolet-visible spectrophotometry and chemiluminescence, respectively. Serum will be isolated by centrifugation and stored at a – 20 ºC freezer within 48-72 hours until analysis. Samples will be treated and centrifuged by a single researcher under standardized conditions. The analysis will be performed in a laboratory in external quality assurance

programs of the Spanish Society of Clinical Chemistry and Molecular Pathology.

2.6.6. Lines 219-220, will the recall list of words be changed for each visit to prevent any carry over between visits? This should be specified.

Answer to reviewer:

In effect, to prevent any carry over between visits, the recall list of words will be changed for each one of the three visits and for each evaluation within the same visit. This has been specified in the text (Line 292, Page 11):

For each one of the three visits and for each evaluation within the same visit, a list of 15 different words from the Rey Auditory Verbal Learning Test and its alternative versions [39-41] will be used to evaluate the immediate verbal memory via immediate recall over three attempts.

2.6.7. Line 221, why is this period "approximately" 10 minutes? Is this likely to be substantially different between trials?

Answer to reviewer:

Delayed verbal memory will be assessed at the end of the cognitive performance evaluation. After conducting a pilot study, we can confirm that this period will be exactly 10 minutes. Therefore, we have introduced this modification in the “cognitive performance evaluation variables” section (Line 295, Page 11):

Delayed verbal memory will be assessed by free recall of the words learnt in the first part of the evaluation after a period of 10 minutes.

2.6.8 Lines 219-221, is this a commonly used test and if so is there a reference that can be provided to substantiate the efficacy of the test?

Answer to reviewer:

In order to assess verbal memory, we have used standardized lists of 15 words from the Rey Auditory Verbal Learning Test and its alternative versions.

We have included this information in the text (Line 292, Page 11):

For each one of the three visits and for each evaluation within the same visit, a list of 15 different words from the Rey Auditory Verbal Learning Test and its alternative versions [39-41] will be used to evaluate the immediate verbal memory via immediate recall over three attempts.

We have also introduced the following references to support this task:

- Rey, A. (1964). L’Examen clinique en psychologie. Paris: Press Universitaire de France.
- Shapiro DM, Harrison DW. Alternate forms of the AVLT: a procedure and test of form equivalency. Arch Clin Neuropsychol. 1990;5(4):405-10.
- Lezak MD. Neuropsychological assessment. 2nd ed. New York: Oxford University Press; 1983.

2.7. Intervention

2.7.1. Lines 232-240, as this study is specifically examining a comparison of GI at breakfast-the GI of the meals should be stated. Additionally the macronutrient composition of the breakfasts should be stated.

Answer to reviewer:

We have included the information about the macronutrient content and glycaemic index of these breakfasts in the methods section (Line 208-220, Page 8):

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

Additionally, we have introduced the information about the energy and glycaemic index in the abstract (methods) as the point 2.1.2 shows.

2.7.2. Line 241, I would recommend that this outlining of the study protocol is moved to earlier in the methods with the detailing of the specific measures afterwards. I would also recommend this might be easier to comprehend as a schematic rather than in written form as currently.

Answer to reviewer:

As noted by the reviewer, we have moved this outlining of the study protocol earlier than the detailing of the specific measures (Line 188, Page 7).

In order to improve its understanding, we have also reworded it and now it appears out of the intervention section as “Study procedures”:

On arrival at the research unit, subjects will be weighed, and their height, waist circumference and hip circumference will be measured. Participants will be seated and remain in this position throughout the visit. After 5 minutes of rest, a peripheral blood pressure measurement will be performed. Immediately, the central blood pressure and hemodynamic parameters will be obtained. Next, cognitive performance will be assessed and fasting blood samples will be collected and central blood pressure and hemodynamic parameters will be determined again. Subjects will be provided with a randomly assigned breakfast to be consumed within 10 minutes. At the first bite, a timer will be started and additional measurements of central blood pressure and hemodynamic parameters will be taken every 15 minutes. Furthermore, another two cognitive performance evaluations and postprandial blood sampling will be completed at 60 and 120 minutes.

2.7.3. Line 242, "Each of the three scheduled visits will last approximately 2 and a half hours", further detail is required here. What time are these visits to occur? What controls are in place for the participants beforehand?

Answer to reviewer:

After performing a pilot study, we can confirm that each of the three scheduled visits will last 2 hours 40 minutes. In addition, these visits will occur between 8:15 am and 10:55 am and participants will be asked to fast for 12 hours overnight prior and to limit their physical activity, alcohol consumption and smoking during the previous 24-48 hours.

We have included this information in the methods section (Line 201, Page 8):

Each of the three scheduled visits will last 2 hours 40 minutes; this will occur between 8:15 am and 10:55 am. Participants will be asked to fast for 12 hours overnight prior and to limit their physical activity, alcohol consumption and smoking during the previous 24-48 hours.

2.7.4 Line 244, what does "clinical" blood pressure mean here?

Answer to reviewer:

It refers to the measurement of the peripheral blood pressure. In order to clarify this subheading, we have reworded it and now reads as follows (Line 265, Page 10):

Peripheral blood pressure.

2.8. Statistical analysis

2.8.1. Lines 268-269, "student t-test for paired data" is to be used? Is this within groups over the timecourse or between groups at specific timepoints?

Answer to reviewer:

Following the suggestions of the reviewer, we have modified the issues raised on the statistical analysis section, remaining as follows (Line 307, Page 12):

The data will be quantitated using the Q of the Cochrane test for qualitative data as well as repeated measures for ANOVA or the Friedman test if the data is non-normally distributed for quantitative data. To compare the differences among the three types of breakfast in quantitative variables, ANOVA test will be used, or Wilcoxon test if the data is non-normally distributed. We will use Least Significant Difference (LSD) test as post hoc analysis and the Chi Square test in qualitative variables. The relationship of quantitative variables to each other will be tested using Pearson's or Spearman's correlation as appropriate. The effect of the interventions can be modified by age, gender, cultural and socioeconomic level, body mass index, lifestyles and last menstruation date. To control the effect of such confounding factors on the study results and to evaluate adequately the effect of the interventions, a multivariate analysis will be performed using the General Lineal Model (GLM) over in basic or extended models. The contrasting hypothesis will establish an alpha risk of 0.05 because the limit of statistical significance. The data will be analysed using the IBM SPSS Statistics for Windows version 23.0 (Armonk, NY: IBM Corp).

2.8.2. Lines 272-273. "the differences in the acute effect of each tested breakfast will be studied by gender" how is this to be done?

Answer to reviewer:

The differences by sex in the response to each breakfast will be analysed. However, the main objective of our study is to determine the acute effect of high versus low GI breakfasts in the variables studied, so we think that this statement could be deleted, since it supposes additional information.

2.8.3. Lines 273-277, "statements are made about the modifying effects of a range of variables upon the study outcomes here, but as this is a paired design this seems of limited importance? Additionally it is stated that the effects of such confounding factors will be controlled, but as there are so many of these variables surely the study is not adequately powered for this?

Answer to reviewer:

To analyse the changes in the dependent variables due to each type of breakfast, we have considered that it would be appropriate to perform a multivariate analysis in two models. This has been described in the statistical analysis section (Line 316, Page 12):

To control the effect of such confounding factors on the study results and to evaluate adequately the effect of the interventions, a multivariate analysis will be performed using the General Lineal Model (GLM) over in basic or extended models.

2.8.4. Will the order effect for trials be examined?

Answer to reviewer:

As you can view at the design and setting section, the order of the three interventions will be determined by a randomization sequence. In this way, its effect for trials will be controlled.

2.9. Methodological limitations

2.9.2. Lines 283-289, see above.

Answer to reviewer:

As noted by the reviewer, these statements do not suppose a limitation in a paired design, so they could be removed.

2.10. Ethical and safety considerations

2.10.1. I would recommend this section is moved to the front of the methods section.

Answer to reviewer:

According to the reviewer's suggestions, we have moved the "ethical and safety considerations" section to the front of the methods (Line 137, Page 5).

2.10.2. Line 299, "None of examinations poses" needs rephrasing.

Answer to reviewer:

As noted by the reviewer above, we have reworded this sentence (Line 146, Page 6):

None of the testing could result in life-threatening risks for the subjects to be enrolled.

2.10.3. Lines 302-303, "with the conditions foreseen" is unclear here. Rephrase.

Answer to reviewer:

As the reviewer's suggests, we have rephrased it and now reads as follows (Line 149, Page 6):

Subject confidentiality will be ensured at all times in accordance with current laws and regulations on personal data protection (LOPD 15/1999 of 13 December) as well as with the conditions contemplated in Act 14/2007 on biomedical research.

2.11. Discussion

2.11.1. Line 311, reference 37 seems like a particularly relevant piece of evidence and this should be referred to in the introduction.

Answer to reviewer:

Following the reviewer's suggestions, this paragraph has been moved to the background section (Line 87, Page 4).

2.11.2. Line 306, as in several other places "proving" is inappropriate here.

Answer to reviewer:

As you can be seen below, this sentence has been reworded and "proving" does not already appear (Line 328, Page 12).

2.11.3. Lines 312-313, "few authors have proven the effect of breakfast on vascular function and hemodynamic parameters 19,20". This wording is unclear, what have these authors actually found?

Answer to reviewer:

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| | As it is detailed on the background section, contrasting results have been reported by Ahuja and Pal [Ahuja KD et al: The American journal of clinical nutrition 2009, 90(2):298-303; Pal S, et al: J Am Coll Nutr 2008, 27(3):387-393]. This specification has been introduced in the mentioned line (Line 338, Page 13):<br><br>Contrasting results have been reported about the effect of breakfast on vascular function and hemodynamic parameters [21, 22].<br><br>2.11.4. Line 314, reduced should be reduce. "Insulin blood levels" should be rephrased.<br><br>Answer to reviewer:<br><br>As the objectives of the study have been reconsidered, we have modified the wording of the results expected and the term “insulin blood levels” has been replaced for “glycaemic response” (Line 339, Page 13):<br><br>In this project, we expect to demonstrate that low glycaemic index breakfast has a favourable effect on measures of vascular function and glycaemic response relative to high glycaemic index.<br><br>2.11.5. Line 315, as insulin will be increased relative to control in the high and low GI trials, this line should clearly indicate that insulin will be lower than high GI, not lower than baseline e.g. reduce blood pressure and insulin concentrations relative to the high GI condition.<br><br>Answer to reviewer:<br><br>As the reviewer suggests, we have reworded this line (Line 339, Page 13):<br><br>In this project, we expect to demonstrate that low glycaemic index breakfast has a favourable effect on measures of vascular function and glycaemic response relative to high glycaemic index.<br><br>2.11.6. Lines 317-321, are these predictions based upon evidence comparing effect of GI from previous studies (e.g. several studies by Lamport et al in 2013/2014) or just predictions?<br><br>Answer to reviewer:<br><br>Based on the results found on previous studies performed on children and adolescents, we hypothesize that similar effects on different cognitive functions could be found in young healthy adults, since their cognitive processes are healthy and not impaired as in diabetic or obese patients. Results to be obtained in this study may help us in understanding how GI can influence on cognitive processes. This information has been stated in the text, and the current paragraph reads as follows (Line 342, Page 13):<br><br>Secondly, based on the findings of previous studies conducted on children and adolescents [26-30, 32], we hypothesize that we will see similar effects in healthy young adults. Thus, we expect that the low glycaemic index breakfast has a positive impact on immediate and delayed verbal memory and verbal fluency, while high glycaemic index breakfast will positively affect attention, processing speed and working memory.<br><br>2.11.7. Lines 322-325 are not clear and should be reworded.<br><br>Answer to reviewer:<br><br>The indicated lines have been reworded for improving their clarity (Line 348, Page 13):<br><br>Therefore, according to our hypothesis, the results of the current study may explain the influence of glycaemic index on cognitive and vascular function. Moreover, given the high worldwide prevalence of cardiovascular diseases and its close relationship with |

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| | cognitive decline, it would be interesting to know how the vascular parameters and cognitive processes are affected by the type of breakfast consumed in regards to the design of novel lifestyle and dietary interventions.<br><br>2.11.8. Lines 325-328, the authors have already stated in lines 318-321 that they expect different positive effects upon different aspects of cognitive performance by the high and low GI breakfasts. Therefore in these final lines they refer to "a breakfast that improves cognitive performance" which type of breakfast is this expected to be? Or is this advocating simply consumption of breakfast as opposed to fasting?<br><br>Answer to reviewer:<br><br>This sentence does not refer to a specific breakfast. Our idea was to state that each breakfast could improve certain cognitive functions so it would be possible to adapt its composition for increasing performance at each type of job.<br><br>In order to clarify it, we have reworded this paragraph (Line 354, Page 13):<br><br>Finally, our results may provide tools for adapting breakfast composition to the tasks that should be performed and improve work performance.<br><br>2.11.9. Line 328, the reference to school here should be removed.<br><br>Answer to reviewer:<br><br>As noted by the reviewer, the reference to school at the end of the discussion has been removed (Line 354, Page 13):<br><br>Finally, our results may provide tools for adapting breakfast composition to the tasks that should be performed and improve work performance.<br><br>2.12. Table 1 and Figure 1 don't seem particularly informative so I would suggest can be removed.<br><br>Answer to reviewer:<br><br>We agree with the reviewer that the Table 1 does not add anything over what is stated in the text, so it could be removed. However, we think that the Figure 1 should not be deleted, since it schematically shows the phases of this crossover clinical trial, improving its understanding. |

**Title:**

Postprandial effect of breakfast glycaemic index on vascular function, glycaemic control and cognitive performance (BGI study): Study protocol for a crossover clinical trial.

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**Abstract:**

***Background:*** Postprandial glycaemic response affects cognitive and vascular function. The acute effect of breakfast glycaemic index on vascular parameters is not sufficiently known. In turn, the influence of breakfasts with different glycaemic index on cognitive performance has been mostly studied in children and adolescents with varying results. Therefore, the purpose of this study is to analyse the postprandial effect of high and low glycaemic index breakfasts on vascular function and cognitive performance and their relationship with postprandial glycaemic response in healthy young adults.

***Methods/Design:*** This is a crossover clinical trial targeted adults (aged 20-40 years, free from cardiovascular disease) selected by consecutive sampling at urban primary care health clinics in Salamanca (Spain). Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ). The postprandial effect will be assessed at 60 and 120 minutes from each breakfast including blood sampling and cognitive performance evaluations. Measurements of

arterial stiffness and central hemodynamic parameters will be taken at -10, 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes.

***Discussion:*** The differences in breakfast glycaemic index could affect vascular parameters, cognitive performance and postprandial glycaemic response with important applications and implications for general population. This provides necessary information for the establishment of new strategies in terms of nutritional education and work performance improvement.

***Trial registration:*** ClinicalTrials.gov Identifier NCT02616276. Registered 19/11/2015.

**Keywords:** Glycaemic index, Postprandial period, Vascular stiffness, Blood glucose, Cognition.

**Background:**

The glycaemic index (GI) is a measure of the speed with which a carbohydrate is absorbed compared to a reference product (pure glucose) [1, 2]. Diets with a high GI increase the risk of diseases related to chronic lifestyles such as type 2 diabetes mellitus [3, 4]. A recent meta-analysis of fourteen prospective studies found that high GI diets are associated with an increased risk of cardiovascular disease (CVD) [5] while a reduction in dietary GI can favourably affect the incidence of coronary disease in women [6]. Low GI diets might reduce the risk of CVD because they decrease postprandial glycaemia with different metabolic effects including differences in insulin sensitivity, circulating lipid concentrations and vascular function [3].

Regarding this latter aspect, the currently accepted gold standard to assess arterial stiffness is carotid-femoral pulse wave velocity (PWV) [7], which has been related to increased morbidity and mortality in both patients with CVD and healthy individuals [8, 9]. Likewise, the augmentation index (AIx) is a measure of wave reflection and arterial stiffness that has been shown to be a predictor of both future cardiovascular events and all-cause mortality [10]. In this way, the Conduit Artery Function Evaluation (CAFE)

study [8] was designed to examine the impact of two different blood pressure (BP) lowering-regimens on derived central aortic pressures. It showed a greater morbidity and mortality and a higher central blood pressure (CBP) and AIx in one of the treatment groups versus the other with no differences in peripheral BP.

In turn, the Lifestyles and Vascular Aging (EVIDENT) study [11] analysed the relationship between lifestyle and arterial aging in a sample of 1553 subjects who were free from cardiovascular disease. We concluded that low GI diets were associated with lower AIx values. In this regard, a reduction in central hemodynamic parameters, AIx and PWV at 60 minutes from food intake has been reported in healthy adults perhaps because of an increase in insulin and/or visceral vasodilatation [12]. Another possible explanation for these findings might be the postprandial hypotension that occurs after a meal due to decreased cortisol secretion and activation of parasympathetic system [13]. For these reasons, although the effects of various macronutrients on vascular function have been explored in a number of studies [14-17], Taylor et al. [12] underlined the importance of analysing the impact of different types of meals on parasympathetic activity, CBP and vascular function parameters.

Of particular interest is the carbohydrate (CHO) content of a meal, which changes postprandial glucose and insulin levels and results in varying AIx reductions in postmenopausal women [18]. Thus, breakfast would play a fundamental role because low and high GI meals eaten in the morning have resulted in better glycaemic control versus eating in the evening [19]. However, despite the fact that breakfast patterns are associated with the metabolic profiles [20], few authors have studied its effect on cardiovascular responses. Ahuja et al. [21] found that a light breakfast (1301 KJ energy) reduced AIx, CBP and BP, and increased heart rate (HR) in adults versus fasting (water). In contrast, a trial aimed to compare the dietary effects of a high GI with a low GI breakfast replacement in obese and overweight individuals reported no differences in BP between breakfasts together with beneficial changes in fasting glucose and insulin levels unaffected [22].

On the other hand, increasing evidence has shown that the postprandial glycaemic response also has a potential impact on cognitive function [23]. Because breakfast is often the first meal before a long working day, there is interest in examining the influence of breakfasts with different GI on cognitive outcomes in healthy young adults.

Cognitive performance may be influenced by many factors including individual differences, socioeconomic status or nutritional and health status [24]. The effect that different GI breakfasts may have on cognitive performance has been studied in people with type 2 diabetes mellitus and obesity, but it is not clear that a specific GI breakfast could benefit cognitive processes in these participants [23, 25]. However, various studies conducted on children have explored the relationship between breakfasts consisting of different GI foods and cognitive functions with contrasting results [24, 26-29]. It appears that a low GI breakfast can benefit the immediate [28-30] and delayed [31] verbal memory as well as sustained attention [30] and verbal fluency [32]. A high GI breakfast may confer benefits for selective attention, processing speed and working memory [32]. In addition, consuming different GI carbohydrates at breakfast could modulate cognitive performance, but this effect requires more study [33].

***Objectives:***

The primary objective of this study is to evaluate the postprandial effect of low and high glycaemic index breakfasts on vascular function as measured by central blood pressure, augmentation index and pulse wave velocity in a sample of healthy young adults. The secondary aim is to assess the postprandial effect of low and high glycaemic index breakfasts on cognitive performance in a sample of healthy young adults. The third goal is to analyse the association between postprandial glycaemic response and vascular function and cognitive performance for high versus low glycaemic index breakfasts.

**Methods/design:**

***Ethical and safety considerations:***

The Clinical Research Ethics Committee of the Health Area of Salamanca ("CREC of Health Area of Salamanca") approved the study on 14 April 2015. The trial is registered in ClinicalTrials.gov with identifier NCT02616276.

*Informed consent:*

Participants must sign the informed consent before inclusion in the study in accordance with the Declaration of Helsinki. Subjects will be informed about the objectives of the study and the risks and benefits of the examinations that they will undergo including the sample collection. None of the testing could result in life-threatening risks for the subjects to be enrolled. Subject confidentiality will be ensured at all times in accordance with current laws and regulations on personal data protection (LOPD 15/1999 of 13 December) as well as with the conditions contemplated in Act 14/2007 on biomedical research.

***Design and setting:***

We designed a controlled crossover clinical trial where each subject will complete three interventions (control breakfast, high glycaemic index breakfast and low glycaemic index breakfast) with a washout period of one week between each trial. The order will be determined by a randomization sequence generated using randomization.com software (http://www.randomization.com) (Figure 1).

*Study setting:*

The study will be conducted on the primary care health area of Salamanca in "La Alamedilla" Research Unit belonging to the Spanish Network for Preventive Activities and Health Promotion (redIAPP) and the Institute for Biomedical Research of Salamanca (IBSAL).

***Study population:***

The subjects will be selected by consecutive sampling in the primary care clinics of urban health centres from Salamanca (Spain) between 2015 and 2016. Because this is a crossover clinical trial, a consecutive sampling will ensure the comparability of interventions.

*Inclusion criteria:*

The study targets young adults aged 20-40 years of both sexes who agree to sign the informed consent.

*Exclusion criteria:*

Subjects will be excluded with a history of cardiovascular events (acute myocardial infarction, stroke, etc.), hypertension, diabetes mellitus, dyslipidaemia, pharmacological treatment for any of these conditions, neurological and/or neuropsychological disease, or the consumption of toxic substances. We will also exclude celiac patients and/or those intolerant to lactose, subjects on a low-calorie and/or low-sodium diet, pregnant women, or those with any other circumstance that the investigators suggest will interfere with the study procedures.

***Sample size:***

The primary outcome variable is change in central augmentation index (AIx). The CAFE study [8] found a reduction in cardiovascular events associated with a decline of 6.5 (5.8, 7.3) points in the Aix. This is the basis of our calculation. The power calculation was a repeated measures design and compared both intervention breakfasts with a control breakfast with an alpha risk of 0.05 and a beta risk of 0.2. The SD was 10 with a correlation coefficient between the initial and final measurement of 0.7. Thus, 40 subjects are required to detect a minimum difference of 5 points in the AIx between two intervention breakfasts. A loss to follow-up of 5% was estimated.

***Study procedures:***

On arrival at the research unit, subjects will be weighed, and their height, waist circumference and hip circumference will be measured. Participants will be seated and remain in this position throughout the visit. After 5 minutes of rest, a peripheral blood pressure measurement will be performed. Immediately, the central blood pressure and hemodynamic parameters will be obtained. Next, cognitive performance will be assessed and fasting blood samples will be collected and central blood pressure and hemodynamic parameters will be determined again. Subjects will be provided with a

randomly assigned breakfast to be consumed within 10 minutes. At the first bite, a timer will be started and additional measurements of central blood pressure and hemodynamic parameters will be taken every 15 minutes. Furthermore, another two cognitive performance evaluations and postprandial blood sampling will be completed at 60 and 120 minutes.

***Intervention:***

Each of the three scheduled visits will last 2 hours 40 minutes; this will occur between 8:15 am and 10:55 am. Participants will be asked to fast for 12 hours overnight prior and to limit their physical activity, alcohol consumption and smoking during the previous 24-48 hours.

*Nutritional composition of each intervention arm:*

1) Control breakfast (BF-1):

This will consist of 350 mL of water served at room temperature.

2) High glycaemic index breakfast (BF-2):

This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

3) Low glycaemic index breakfast (BF-3):

This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

*Blinding strategy:*

Because of the nature of the interventions, participants and research staff cannot be blinded. However, those responsible for statistical analysis will be blinded to the interventions.

***Variables and measurement instruments:***

*Sociodemographic variables:*

At the time of study entry and prior to the first intervention visit, data on age, gender, marital status, educational level, and occupation will be collected.

*Lifestyle-related variables:*

They will be collected at the time of study entry and prior to the first intervention visit.

1) Smoking:

This will be measured using a questionnaire on smoking history and tobacco consumption pattern.

2) Alcohol consumption:

This will be measured using a questionnaire on alcohol consumption in the past 7 days specifying drinks and their volumes.

3) Regular diet:

This will be assessed with Diet Quality Index (DQI) [34], which is a validated questionnaire that records the frequency of food intake and assigns a score ranging from 18 to 54 points.

4) Regular physical activity:

This will be evaluated with the International Physical Activity Questionnaire (IPAQ) [35] in its short version that is validated for a Spanish population. It assesses activity in the last 7 days differentiating between three types (walking, moderate-intensity and vigorous-intensity activity) and, according to energy expenditure estimated for each of them (3.3, 4, and 8 MET, respectively). It allows the MET-minutes/week to be calculated and to classify subjects according to three activity levels (low, intermediate and high).

*Female-specific variables:*

The date of the last menstruation will be recorded because it may impact the study variables.

*Anthropometric variables:*

The height will be measured with the subject standing barefoot recording the average of two readings rounded to the nearest centimetre using a portable system (Seca 222; Medical scale and measurement system, Birmingham, United Kingdom).

The body weight will be measured with the subject barefoot and wearing light clothing, recording the average of two readings rounded to 100 g using a standard electronic balance (Seca 770; Medical scale and measurement system, Birmingham, United Kingdom) that is properly calibrated (precision ± 0.1 kg).

Following the recommendations of the Spanish Society for the Study of Obesity (SEEDO) [36], the waist circumference will be measured in duplicate at level of the midpoint between the last rib and the iliac crest with the subject standing without clothing using a flexible tape parallel to the floor after inspiration. Hip circumference will be similarly measured at the level of the trochanters.

*Peripheral blood pressure:*

Three measurements of systolic (SBP) and diastolic (DBP) blood pressure will be performed using the average of the last two with a validated OMROM M10-IT model sphygmomanometer (Omron Healthcare, Kyoto, Japan). They will be made on the participants' dominant arm in a seated position after at least 5 minutes of rest with a cuff of appropriate size as determined by measurement of the upper-arm circumference and following the recommendations of the European Society of Hypertension [37].

*Arterial stiffness and central hemodynamic parameters:*

The Mobil-O-Graph® device [38] will be used to estimate cardiac output and total peripheral vascular resistance and to measure central blood pressure, pulse wave velocity, reflection coefficient and augmentation index. This is affected by heart rate (HR) so its values will be corrected to a HR of 75 bpm. This device will be scheduled to

perform continuous measurements at -10, 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes with the subject sitting and resting his arm on a rigid surface.

*Laboratory variables:*

At the time of study entry and prior to the first intervention visit, fasting plasma creatinine, serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides values will be determined using standard enzymatic automated methods.

During each study visit, three cannula blood samples will be collected at -10, 60 and 120 minutes to measure serum glucose and insulin levels by ultraviolet-visible spectrophotometry and chemiluminescence, respectively. Serum will be isolated by centrifugation and stored at a – 20 ºC freezer within 48-72 hours until analysis. Samples will be treated and centrifuged by a single researcher under standardized conditions. The analysis will be performed in a laboratory in external quality assurance programs of the Spanish Society of Clinical Chemistry and Molecular Pathology.

*Cognitive performance evaluation variables:*

For each one of the three visits and for each evaluation within the same visit, a list of 15 different words from the Rey Auditory Verbal Learning Test and its alternative versions [39-41] will be used to evaluate the immediate verbal memory via immediate recall over three attempts. Delayed verbal memory will be assessed by free recall of the words learnt in the first part of the evaluation after a period of 10 minutes. Phonological fluency will be explored by enumerating for one minute as many words as possible starting with different letters [42]. The Trail Making Test A will be used to assess attention and processing speed, while executive functions will be explored using the Trail Making Test B [43]. Working memory will be traced with the WAIS Digit Span Backward test [44]. Finally, sustained and selective attention, executive functions and processing speed will be explored with the Stroop test [45].

***Statistical analysis****:*

The normal distribution of variables will be verified using a Kolmogorov-Smirnov test. Quantitative variables will be displayed as the mean ± standard deviation if normally

distributed or as the median (interquartile range) if asymmetrically distributed. The qualitative variables will be expressed as frequencies. The data will be quantitated using the Q of the Cochrane test for qualitative data as well as repeated measures for ANOVA or the Friedman test if the data is non-normally distributed for quantitative data. To compare the differences among the three types of breakfast in quantitative variables, ANOVA test will be used, or Wilcoxon test if the data is non-normally distributed. We will use Least Significant Difference (LSD) test as post hoc analysis and the Chi Square test in qualitative variables. The relationship of quantitative variables to each other will be tested using Pearson's or Spearman's correlation as appropriate. The effect of the interventions can be modified by age, gender, cultural and socioeconomic level, body mass index, lifestyles and last menstruation date. To control the effect of such confounding factors on the study results and to evaluate adequately the effect of the interventions, a multivariate analysis will be performed using the General Lineal Model (GLM) over in basic or extended models. The contrasting hypothesis will establish an alpha risk of 0.05 because the limit of statistical significance. The data will be analysed using the IBM SPSS Statistics for Windows version 23.0 (Armonk, NY: IBM Corp).

***Methodological limitations:***

This study follows all the CONSORT recommendations, but participants cannot be blinded due to the intervention characteristics. However, the investigator who analyses the data will be blinded.

The acute effect of the high or low glycaemic index breakfast on the study variables may be influenced by age, gender and conditions that cause endothelial dysfunction such as hypertension, diabetes or dyslipidaemia. Therefore, the study population will comprise young subjects, of both sexes, free from cardiovascular disease, hypertension, diabetes or dyslipidaemia.

**Discussion:**

There is accumulating evidence that postprandial glycaemic response is associated with cognitive and vascular function [18, 23]. To our knowledge, the postprandial effects of high and low glycaemic index breakfasts on vascular parameters and cognitive performance have not been previously concurrently investigated.

Contrasting results have been reported about the effect of breakfast on vascular function and hemodynamic parameters [21, 22]. In this project, we expect to demonstrate that low glycaemic index breakfast has a favourable effect on measures of vascular function and glycaemic response relative to high glycaemic index.

Secondly, based on the findings of previous studies conducted on children and adolescents [26-30, 32], we hypothesize that we will see similar effects in healthy young adults. Thus, we expect that the low glycaemic index breakfast has a positive impact on immediate and delayed verbal memory and verbal fluency, while high glycaemic index breakfast will positively affect attention, processing speed and working memory.

Therefore, according to our hypothesis, the results of the current study may explain the influence of glycaemic index on cognitive and vascular function. Moreover, given the high worldwide prevalence of cardiovascular diseases and its close relationship with cognitive decline, it would be interesting to know how the vascular parameters and cognitive processes are affected by the type of breakfast consumed in regards to the design of novel lifestyle and dietary interventions.

Finally, our results may provide tools for adapting breakfast composition to the tasks that should be performed and improve work performance.

**List of abbreviations used:**

BGI: Breakfast glycaemic index; GI: Glycaemic index; CVD: Cardiovascular disease; PWV: Pulse wave velocity; AIx: Augmentation index; CAFE: Conduit artery function evaluation; BP: Blood pressure; CBP: Central blood pressure; EVIDENT: Lifestyles and vascular aging; CHO: Carbohydrate; HR: Heart rate; CREC: Clinical research ethics

committee; LOPD: Legislation on personal data protection; REDIAPP: Network for preventive activities and health promotion; IBSAL: Institute for biomedical research of Salamanca; BF: Breakfast; DQI: Diet quality index; IPAQ: Physical activity questionnaire; MET: Metabolic equivalent; SEEDO: Spanish Society for the Study of Obesity; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; WAIS: Wechsler adults intelligence scale.

**Competing interests:**

The authors declare that they have no competing interests.

**Authors' contributions:**

JIR-R, NS-A and LG-O conceived and designed the study. NS-A, JIR-R and SM-S drafted the protocol of the study. LG-O, MCP-A, MAG-M and SM-S provided methodological and statistical expertise. JIR-R is responsible for study management, staff training, and supervision. NS-A manages day-to-day study responsibilities, including monitoring recruitment, and liaising with recruitment sites. NS-A, BS-S and RA-D participates in data collection. NS-A and JIR-R wrote the manuscript. LG-O made the final review. All the authors have read the draft critically, contribute and have approved the final text.

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**Figure legends:**

Figure 1; Flow-chart of BGI study; (BF: Breakfast).

Figure 1

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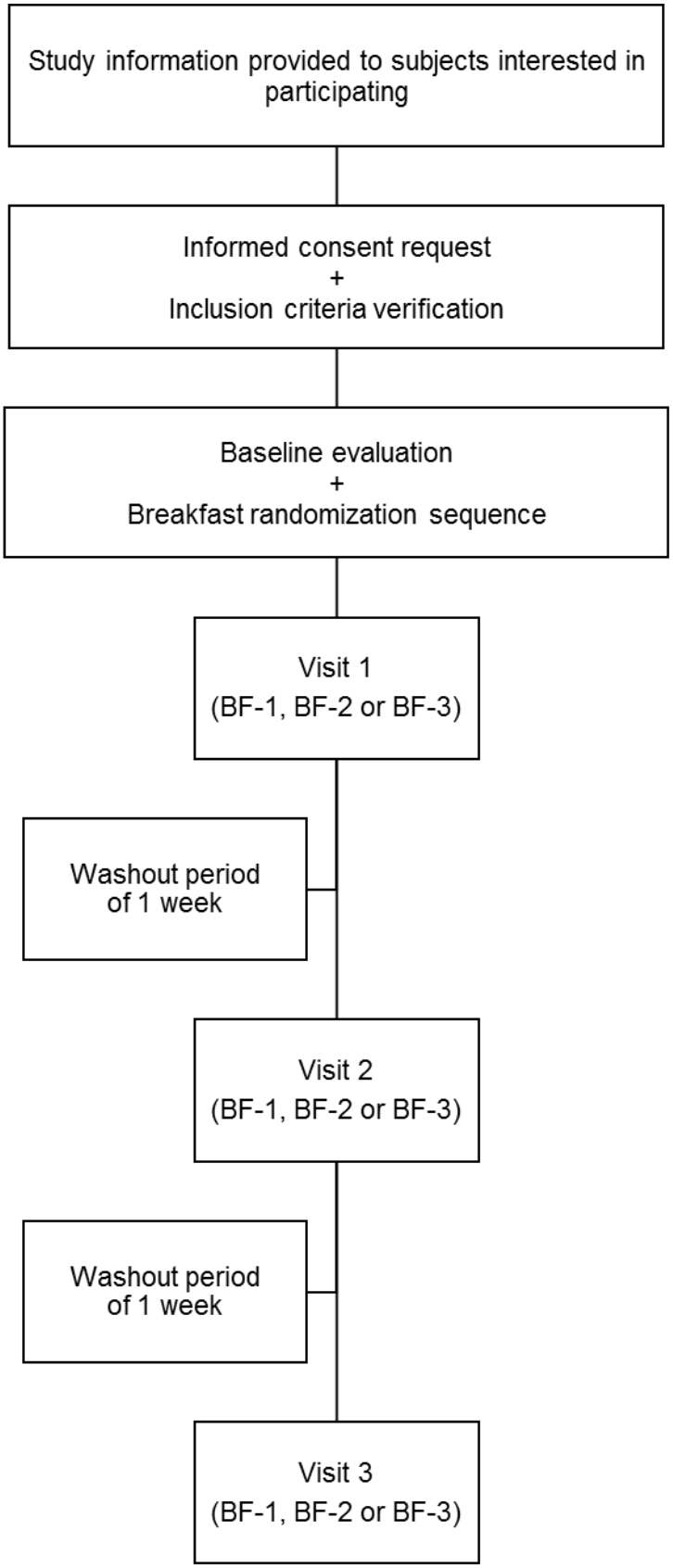

English Editing Certificate

Dear Editor of Trials:

Thank you for your help in reviewing this STUDY PROTOCOL for consideration of publication in Trials.

Following the suggestions of the reviewers, we enclose a new version of our manuscript entitled: “Postprandial effect of breakfast glycaemic index on vascular function, glycaemic control and cognitive performance (BGI study): Study protocol for a crossover clinical trial” TRLS-D-16-00075**,** together with replies to all the issues raised.

GENERAL COMMENTS:

As the reviewers suggest, the background and discussion sections have been restructured to clarify the specific novel aspects of our study, some of the methods to be employed have been detailed and the entire manuscript has been reviewed by an English language professional (English editing certificate attached as supplementary material). These changes are shown underlined in the current study protocol. We think that its understanding is better now. We therefore believe that its interest has increased considerably.

We look forward to hearing from you. If you have any additional request or need any information, please contact us.

Sincerely:

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***Reviewer #1:*** **A very interesting paper investigating the role of GI at breakfast on cognitive function, vascular function and insulineamia.**

Answer to reviewer:

First, thank you for your work in reviewing this study protocol. Your contributions and suggestions will improve the understanding of the text.

**The manuscript itself is a trial design only. All elements use standard protocols but this paper has decided to combine them to research its intended study aims. Overall the paper is of good quality, but a number of clarifications are required before publication. I have listed specific concerns below**:

**1. The introduction is very clunky and needs to be restructured. There are 3 distinctive sections that are discussed but they would benefit from being linked together more clearly. Currently they read as if 3 standalone studies are stuck together. I would recommend the authors provide a clearer rationale for taking all 3 measures together at the same time in one study in the introduction.**
**In addition, the authors have reported data from studies using children and adolescent participants (lines 107:118). It would be more beneficial to explore studies in adult populations.**

**1.1. The introduction is very clunky and needs to be restructured. There are 3 distinctive sections that are discussed but they would benefit from being linked together more clearly. Currently they read as if 3 standalone studies are stuck together. I would recommend the authors provide a clearer rationale for taking all 3 measures together at the same time in one study in the introduction.**

Answer to reviewer:

Following the suggestions of the reviewer, the background has been restructured and its three distinctive sections have been linked together more clearly (Line 68, Page 3):

The glycaemic index (GI) is a measure of the speed with which a carbohydrate is absorbed compared to a reference product (pure glucose) [1, 2]. Diets with a high GI increase the risk of diseases related to chronic lifestyles such as type 2 diabetes mellitus [3, 4]. A recent meta-analysis of fourteen prospective studies found that high GI diets are associated with an increased risk of cardiovascular disease (CVD) [5] while a reduction in dietary GI can favourably affect the incidence of coronary disease in women [6]. Low GI diets might reduce the risk of CVD because they decrease postprandial glycaemia with different metabolic effects including differences in insulin sensitivity, circulating lipid concentrations and vascular function [3].
Regarding this latter aspect, the currently accepted gold standard to assess arterial stiffness is carotid-femoral pulse wave velocity (PWV) [7], which has been related to increased morbidity and mortality in both patients with CVD and healthy individuals [8, 9]. Likewise, the augmentation index (AIx) is a measure of wave reflection and arterial stiffness that has been shown to be a predictor of both future cardiovascular events and all-cause mortality [10]. In this way, the Conduit Artery Function Evaluation (CAFE) study [8] was designed to examine the impact of two different blood pressure (BP) lowering-regimens on derived central aortic pressures. It showed a greater morbidity and mortality and a higher central blood pressure (CBP) and AIx in one of the treatment groups versus the other with no differences in peripheral BP.
In turn, the Lifestyles and Vascular Aging (EVIDENT) study [11] analysed the relationship between lifestyle and arterial aging in a sample of 1553 subjects who were free of cardiovascular disease. We concluded that low GI diets were associated with

lower AIx values. In this regard, a reduction in central hemodynamic parameters, AIx and PWV at 60 minutes from food intake has been reported in healthy adults perhaps because of an increase in insulin and/or visceral vasodilatation [12]. Another possible explanation for these findings might be the postprandial hypotension that occurs after a meal due to decreased cortisol secretion and activation of parasympathetic system [13]. For these reasons, although the effects of various macronutrients on vascular function have been explored in a number of studies [14-17], Taylor et al. [12] underlined the importance of analysing the impact of different types of meals on parasympathetic activity, CBP and vascular function parameters.

Of particular interest is the carbohydrate (CHO) content of a meal, which changes postprandial glucose and insulin levels and results in varying AIx reductions in postmenopausal women [18]. Thus, breakfast would play a fundamental role because low and high GI meals eaten in the morning have resulted in better glycaemic control versus eating in the evening [19]. However, despite the fact that breakfast patterns are associated with the metabolic profiles [20], few authors have studied its effect on cardiovascular responses. Ahuja et al. [21] found that a light breakfast (1301 KJ energy) reduced AIx, CBP and BP, and increased heart rate (HR) in adults versus fasting (water). In contrast, a trial aimed to compare the dietary effects of a high GI with a low GI breakfast replacement in obese and overweight individuals reported no differences in BP between breakfasts together with beneficial changes in fasting glucose and insulin levels unaffected [22].

On the other hand, increasing evidence has shown that the postprandial glycaemic response also has a potential impact on cognitive function [23]. Because breakfast is often the first meal before a long working day, there is interest in examining the influence of breakfasts with different GI on cognitive outcomes in healthy young adults. Cognitive performance may be influenced by many factors including individual differences, socioeconomic status or nutritional and health status [24]. The effect that different GI breakfasts may have on cognitive performance has been studied in people with type 2 diabetes mellitus and obesity, but it is not clear that a specific GI breakfast could benefit cognitive processes in these participants [23, 25]. However, various studies conducted on children have explored the relationship between breakfasts consisting of different GI foods and cognitive functions with contrasting results [24, 26-29]. It appears that a low GI breakfast can benefit the immediate [28-30] and delayed [31] verbal memory as well as sustained attention [30] and verbal fluency [32]. A high GI breakfast may confer benefits for selective attention, processing speed and working memory [32]. In addition, consuming different GI carbohydrates at breakfast could modulate cognitive performance, but this effect requires more study [33].

**1.2. In addition, the authors have reported data from studies using children and adolescent participants (lines 107:118). It would be more beneficial to explore studies in adult populations.**

Answer to reviewer:

As far as we know, this is the first study that assesses the postprandial effect of different types of breakfasts on cognitive performance in healthy young adults. The studies developed to date have been performed on samples of children and adolescent participants, due to the importance that cognitive functions have on academic development. Knowing the cognitive processes involved in the different cognitive functions studied (verbal and working memories, executive functions, attention, etc.) and the results found in children and adolescents, we have hypothesized that revealing results about this relationship could be found if we studied it in healthy young adults. However, this relationship has been previously studied in samples of people with type 2 diabetes and obesity. For this reason, as you can be seen above, we have introduced a mention to these studies in the background section (Line 116, Page 5).

**2. I have a number of concerns regarding the methodology which need to be clarified:**

**2.1. Can the authors provide a rationale for measuring insulineamia at only 10, 60+ and 120+ minutes? Previous research investigating glucose and insulin response would encourage regular measurements at -5, 0, 15, 30, 45, 60, 90 and 120 mins (Henry et al; FAO/WHO).**

Answer to reviewer:

We are aware of additional blood samples would improve the interpretation of the glycaemic response to consumption of each breakfast. However, we have a limited funding and we consider the glycaemic response as an intermediate variable between vascular function and cognitive performance. Because of that, we have established these three time point's blood samples.

**2.2. Can the authors guarantee that all measurements will be completed at the assigned time there appears to be several measures collected at once which might result in a measure being taken later or earlier predicted?**

Answer to reviewer:

We have performed a pilot study and we can guarantee that all measurements will be completed at the assigned time.

**2.3. Would the measures interfere with each other?**

Answer to reviewer:

The pilot study previously mentioned was performed with the objective of preventing the interference between measurements collected at once. In this sense, cognitive performance evaluations will be exactly completed at indicated minutes, cannula blood samples are planned to be collected next and Mobil-O-Graph® measurements will be scheduled to be performed automatically after.

**2.4. Can the authors provide a rationale for the choice of breakfast foods?**

Answer to reviewer:

The main objective of this study is to determine the effect of high GI breakfast versus low GI in the variables studied. That has been the reason for the choice of these products and not others.

**2.5. Can the authors provide a rationale for providing chocolate as part of the breakfast? Are there more 'traditional' breakfasts that would be more suitable? Or is this representative of a typical breakfast in their area?**

Answer to reviewer:

Most of these products are consumed typically in the breakfast while others are not. The reason for providing chocolate as part of breakfast is its low GI. However, as indicated by the reviewer, a possible and interesting work could be to evaluate the effect of typical breakfast of a region or area as may be the Mediterranean breakfast. But we could discuss it in a future study.

**2.6. More specifically can they confirm the GI values of the breakfasts and provide a comprehensive nutritional breakdown of the foods (energy, carbohydrate etc)**

Answer to reviewer:

The nutritional breakdown of the different breakfast is showed in the following table:

| Type of Breakfast | Glycaemic index, % | Energy, Kcal/KJ | Carbohydrates, % | Protein, % | Fat, % | Fibre, g |
|---|---|---|---|---|---|---|
| High Glycaemic index | 64.0 | 368 /1541 | 91.5 | 5.0 | 2.5 | 1.63 |
| Low Glycaemic index | 29.4 | 356 /1489 | 35.5 | 10.9 | 50.4 | 6.00 |

We have included the information about the macronutrient content and glycaemic index of these breakfasts in the methods section (Line 208-220, Page 8):

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

Additionally, we have introduced the information about the energy and glycaemic index in the abstract (methods) (Line 47, Page 2):

Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ).

**2.7. It is suggested that the authors ensure participants limit their physical activity; alcohol and smoking at least 24 hours before each test. There is substantial evidence to suggest that these components alter glucose and insulin measurements up to 24 or 48 hours.**

Answer to reviewer:

Taking into account the suggestions of the reviewer, the participants will be asked to limit their physical activity, alcohol consumption and smoking during 24-48 hours before each intervention visit.

We have included this information in the methods section (Line 201, Page 8):

Each of the three scheduled visits will last 2 hours 40 minutes and it will occur between 8:15 am and 10:55 am. Participants will be asked to fast for 12 hours overnight prior and to limit their physical activity, alcohol consumption and smoking during the previous 24-48 hours.

**2.8. When and how long after breakfast will the Trail Making Test be completed?**

Answer to reviewer:

The length of the cognitive performance evaluation will be 10 minutes and Trail Making Test will be scheduled at minute 5. Therefore, it will be completed 5 minutes before breakfast and 55 and 115 minutes after this.

**Statistical analysis – The authors indicate that an independent t-test will be conducted to determine differences between breakfasts, however there are three arms (control, High GI and Low GI) and therefore surely a ANOVA is more suitable? This could then be stratified by gender, age, SES etc. as indicated (line 274)**

Answer to reviewer:

We have changed the statistical analysis section, including some new paragraphs and reorganizing others, and now it reads as follows (Line 304, Page 11):

The normal distribution of variables will be verified using a Kolmogorov-Smirnov test. Quantitative variables will be displayed as the mean ± standard deviation if normally distributed or as the median (interquartile range) if asymmetrically distributed. The qualitative variables will be expressed as frequencies. The data will be quantitated using the Q of the Cochrane test for qualitative data as well as repeated measures for ANOVA or the Friedman test if the data is non-normally distributed for quantitative data. To compare the differences among the three types of breakfast in quantitative variables, ANOVA test will be used, or Wilcoxon test if the data is non-normally distributed. We will use Least Significant Difference (LSD) test as post hoc analysis and the Chi Square test in qualitative variables. The relationship of quantitative variables to each other will be tested using Pearson's or Spearman's correlation as appropriate. The effect of the interventions can be modified by age, gender, cultural and socioeconomic level, body mass index, lifestyles and last menstruation date. To control the effect of such confounding factors on the study results and to evaluate adequately the effect of the interventions, a multivariate analysis will be performed using the General Lineal Model (GLM) over in basic or extended models. The contrasting hypothesis will establish an alpha risk of 0.05 because the limit of statistical significance. The data will be analysed using the IBM SPSS Statistics for Windows version 23.0 (Armonk, NY: IBM Corp).

***Reviewer #2*: General comments to the Authors: The manuscript describes a study that would be of relevant to readers interested in clinical nutrition. In particular, the breakfast meal is topic generating significant interest of late and therefore this study would add to this developing body of literature. I do however think that the specific novel aspects of this study needs to be clarified more explicitly in the introduction and discussion sections of this manuscript. In addition, some of the methods employed are not explained in sufficient detail. In particular, the protocol used for measurement of blood pressure and the protocol used for collection, treatment, storage and analysis of blood samples.**
**There are also numerous examples in the manuscript of English language errors. I have highlighted some of these below, but I would advise the authors to seek assistance from an English language professional to improve the readability of the manuscript.**
**I have provided specific comments on each section of the manuscript below, along with a couple of notes about the protocol, which I hope the authors find useful.**

Answer to reviewer:

First, thank you for your work in reviewing this study protocol. Your contributions and suggestions will improve the understanding of the text.

**Specific comments to the Authors:**

**1. Title**

**1.1. As glucose and insulin is being assessed, consider changing 'insulinemia' to 'glycaemic control', as this incorporates the glucose and insulin components of the study.**

Answer to reviewer:

As the reviewer suggests, we have changed “insulinemia” to “glycaemic control” in the title (Line 2, Page 1):

Postprandial effect of breakfast glycaemic index on vascular function, glycaemic control and cognitive performance (BGI study): Study protocol for a crossover clinical trial.

**2. Abstract**

**2.1. Lines 30-36: The background section needs to articulate more explicitly the novel aspect of the study. It is clear that there are several aspects to this study, but why they are being investigated concurrently is not clear.**

Answer to reviewer:

Following the reviewer’s suggestions, this section has been reworded to articulate more explicitly the novel aspect of the study (Line 38, Page 2):

Postprandial glycaemic response affects cognitive and vascular function. The acute effect of breakfast glycaemic index on vascular parameters is not sufficiently known. In turn, the influence of breakfasts with different glycaemic index on cognitive performance has been mostly studied in children and adolescents with varying results. Therefore, the purpose of this study is to analyse the postprandial effect of high and

low glycaemic index breakfasts on vascular function and cognitive performance and their relationship with postprandial glycaemic response in healthy young adults.

**2.2. Line 41: I think 'black chocolate' is generally referred to as 'dark chocolate'.**

Answer to reviewer:

We have replaced the term “black chocolate” for “dark chocolate” in the abstract and the methods:

(Line 47, Page 2): Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ).

(Line 215, Page 8): This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

**2.3. Lines 46-47: I don't think the '+' is required here as it is understood that times greater than 0 will automatically be positive. The '10' should remain though.**

Answer to reviewer:

Taking into account the suggestion of the reviewer, we have deleted the “+” on the indicated line (Line 54, Page 2):

Measurements of arterial stiffness and central hemodynamic parameters will be taken at -10, 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes.

**3. Background**

**3.1. This section requires some alteration as it is not made clear what the novel aspects of this study are. In addition, there are several examples of grammar mistakes and poor English (more than is mentioned below) that need to be corrected.**

Answer to reviewer:

This section has been restructured to clarify the specific novel aspects of the current study. In addition, an English language professional has reviewed the entire manuscript. Thus, we attach the English editing certificate as supplementary material.

**3.1.1. Line 77: I think more information is required than just 'CAFÉ sub-study' as this does not describe what was done.**

Answer to reviewer:

The information required by the reviewer has been included in the text (Line 82, Page 3):

In this way, the Conduit Artery Function Evaluation (CAFE) study [8] was designed to examine the impact of two different blood pressure (BP) lowering-regimens on derived central aortic pressures. It showed a greater morbidity and mortality and a higher central blood pressure (CBP) and AIx in one of the treatment groups versus the other with no differences in peripheral BP.

**3.1.2. Line 80: Reword to 'particularly 60 min after ingestion' or similar.**

Answer to reviewer:

Following the reviewer's suggestion, we have modified this phrasing and now it reads as follows (Line 90, Page 4):

In this regard, a reduction in central hemodynamic parameters, AIx and PWV at 60 minutes from food intake has been reported in healthy adults perhaps because of an increase in insulin and/or visceral vasodilatation [12].

**3.1.3. Line 80-82: This is unclear and needs rewording.**

Answer to reviewer:

These statements come from the cohort study by Zanasi et al. [Zanasi A et al: J Hypertens 2012, 30(11):2125-2132], which assesses the prevalence and the prognostic power of postprandial hypotension in ambulatory hypertensive elderly patients without overt heart diseases. However, the background has been restructured to clarify the specific novel aspects of the current study, and we have thought that these sentences could be removed.

Thus, now the paragraph that included this information reads as follows (Line 92, Page 4):

Another possible explanation for these findings might be the postprandial hypotension that occurs after a meal due to decreased cortisol secretion and activation of parasympathetic system [13].

**3.1.4. Line 82: A decrease in blood pressure is the same as hypotension, so for consistency just use one or the other.**

Answer to reviewer:

As you can be seen above, now we only use the term "hypotension".

**3.1.5. Line 85: The reference cited here was a short (4 h) intervention study investigating postprandial effects of MUFA and SFA meals. I fail to see how this study could determine that BP variation after breakfast is an independent risk factor for mortality.**

Answer to reviewer:

This statement also comes from the cohort study by Zanasi et al. [Zanasi A et al: J Hypertens 2012, 30(11):2125-2132]. However, the background has been restructured

to clarify the specific novel aspects of the current study, and we have thought that this sentence could be removed.

**3.1.6. Lines 93-95: Needs rewording**

Answer to reviewer:

We have reworded these statements (Line 99, Page 4):

Of particular interest is the carbohydrate (CHO) content of a meal, which changes postprandial glucose and insulin levels and results in varying AIx reductions in postmenopausal women [18].

**3.1.7. Line 94: What is meant by 'different reductions'? Please clarify this.**

Answer to reviewer:

“Different reductions” means that varying AIx reductions have been obtained. This has been clarified in the text (Line 99, Page 4):

Of particular interest is the carbohydrate (CHO) content of a meal, which changes postprandial glucose and insulin levels and results in varying AIx reductions in postmenopausal women [18].

**3.1.8. Line 98: Please explain what is meant by the 'variability of CHO content' in breakfast. Generally, traditional breakfast foods tend to be high carbohydrate, so please explain this statement.**

Answer to reviewer:

As noted by the reviewer, breakfast usually has an important load of carbohydrates. However, there can be variability in the glycaemic index of these carbohydrates. This was the idea that we wanted to express.

Nevertheless, as the background has been reworded to articulate more explicitly the novel aspect of the study, we have considered that this statement could be removed, and now the following sentence appears in its place (Line 101, Page 4):

Thus, breakfast would play a fundamental role because low and high GI meals eaten in the morning have resulted in better glycaemic control versus eating in the evening [19].

**3.1.9. Lines 98-104: Several grammar and punctuation errors are present in this paragraph. Please review this.**

Answer to reviewer:

All the errors highlighted have been reviewed. Moreover, we have sought assistance from an English language professional to improve the readability of the manuscript. Thus, we attach the English editing certificate as supplementary material.

**3.1.10. Line 101: HOMA should be defined, but this simply refers to homeostatic model assessment, so perhaps HOMAIR is more accurate. In addition, this is a fasting measure of insulin resistance, so I'm unsure how this can be affected by breakfast. If this is meant as a chronic effect of consuming a low GI breakfast, this needs to be made clear.**

Answer to reviewer:

The main objective of our study is to determine the acute effect of high versus low GI breakfasts in the variables studied, so it is out of context to consider HOMA in the text. For that reason, we have reworded the paragraph referred to this index (Line 17, Page 4):

In contrast, a trial aimed to compare the dietary effects of a high GI with a low GI breakfast replacement in obese and overweight individuals reported no differences in BP between breakfasts together with beneficial changes in fasting glucose and insulin levels unaffected [22].

**3.1.11. Line 102: 'proven' is strong wording here. Consider revising.**

Answer to reviewer:

Following the suggestions of the reviewer, this line has been reworded (Line 105, Page 4):

Ahuja et al. [21] found that a light breakfast (1301 KJ energy) reduced AIx, CBP and BP, and increased heart rate (HR) in adults versus fasting (water).

**3.1.12. Line 102: What is meant by a 'light' breakfast? Please provide energy content to clarify this.**

Answer to reviewer:

We have included in this sentence the energy content of the breakfast, and now it reads as follows (Line 105, Page 4):

Ahuja et al. [21] found that a light breakfast (1301 KJ energy) reduced AIx, CBP and BP, and increased heart rate (HR) in adults versus fasting (water).

**3.1.13. Lines 105-106: Please revise this sentence.**

Answer to reviewer:

This sentence has been revised and its wording modified (Line 111, Page 5):

On the other hand, increasing evidence has shown that the postprandial glycaemic response also has a potential impact on cognitive function [23]. Because breakfast is often the first meal before a long working day, there is interest in examining the influence of breakfasts with different GI on cognitive outcomes in healthy young adults.

**3.1.14. Line 114: consider changing to 'reporting contrasting results'**

Answer to reviewer:

Following the suggestions of the reviewer, we have changed this sentence (Line 119, Page 5):

However, various studies conducted on children have explored the relationship between breakfasts consisting of different GI foods and cognitive functions with contrasting results [24, 26-29].

**3.1.15. Line 114-118: Please revise this section. I'm not sure that significant is required in either of these sentences.**

Answer to reviewer:

As the reviewer suggests, this section has been revised and its wording modified (Line 122, Page 5):

It appears that a low GI breakfast can benefit the immediate [28-30] and delayed [31] verbal memory as well as sustained attention [30] and verbal fluency [32]. A high GI breakfast may confer benefits for selective attention, processing speed and working memory [32].

**3.1.16. Line 125: Remove 'and last'.**

Answer to reviewer:

We have removed “and last” in this sentence (Line 133, Page 5):

The third goal is to analyse the association between postprandial glycaemic response and vascular function and cognitive performance for high versus low glycaemic index breakfasts.

**4. Methods**

**4.1. More information is required on some of the measures to be taken. These are detailed below. Again, several grammatical errors are present in this section which requires revision.**

Answer to reviewer:

The information required by the reviewer on some of the measures to be taken has been detailed below. In addition, an English language professional has reviewed the entire manuscript. Thus, we attach the English editing certificate as supplementary material.

**4.1.1. Line 186: Change 'women' to female.**

Answer to reviewer:

We have changed “women” to “female” and now this subheading reads as follows (Line 249, Page 9):

Female-specific variables.

**4.1.2. Lines 187-189: Ideally stage of menstrual cycle should be controlled. This can be done by having ~1 month between trials (dependant on duration of menstrual cycle) and testing subjects between ~5-12 days after start of menstruation. Alternatively, only recruiting female subject on a monophasic contraceptive pill, and ensuring testing occurs in the active 21 day period.**

Answer to reviewer:

We agree with the reviewer’s assessment that the stage of menstrual cycle should ideally be controlled. However, we have established a washout period of one week

between each intervention for all the participants. Thus, the last menstruation date will be used as control variable on the study results for evaluating adequately the effect of the interventions.

**4.1.3. Lines 197-200 Is waist and hip circumference measured in duplicate? This should be stated as the variation of circumference is likely to be larger than weight, which is measured in duplicate, as stated on line 194.**

Answer to reviewer:

Waist and hip circumference will be measured in duplicate, following the recommendations of the Spanish Society for the Study of Obesity (SEEDO) [Salas-Salvado J et al: Med Clin (Barc) 2007, 128:184-196; quiz 181 p following 200].

We have stated this in the text (Line 260, Pag 10):

Following the recommendations of the Spanish Society for the Study of Obesity (SEEDO) [36], the waist circumference will be measured in duplicate at level of the midpoint between the last rib and the iliac crest with the subject standing without clothing using a flexible tape parallel to the floor after inspiration. Hip circumference will be similarly measured at the level of the trochanters.

**4.1.4. Lines 201-211: What procedures will be followed before measures of blood pressure are taken? Will subjects be seated, standing, supine etc? Will there be a period of rest before measurement? This information need to be included here.**

Answer to reviewer:

Following the reviewer's suggestions, we have added a new paragraph, which includes this information (Line 268, Page 10):

They will be made on the participants' dominant arm in a seated position after at least 5 minutes of rest with a cuff of appropriate size as determined by measurement of the upper-arm circumference and following the recommendations of the European Society of Hypertension [37].

**4.1.5 Line 211: As above, remove the '+' here.**

Answer to reviewer:

As the reviewer suggests, we have removed the "+" on the indicated line (Line 277, Page 10):

This device will be scheduled to perform continuous measurements at -10, 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes with the subject sitting and resting his arm on a rigid surface.

**4.1.6. Lines 212-217: How are blood samples collected, treated, centrifuged and stored? How will blood samples be analysed?**

Answer to reviewer:

As the information provided in this section was insufficient, we have detailed all the issues raised by the reviewer in the text (Line 281, Page 11):

At the time of study entry and prior to the first intervention visit, fasting plasma creatinine, serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides values will be determined using standard enzymatic automated methods.
During each study visit, three cannula blood samples will be collected at -10, 60 and 120 minutes to measure serum glucose and insulin levels by ultraviolet-visible spectrophotometry and chemiluminescence, respectively. Serum will be isolated by centrifugation and stored at a – 20 ºC freezer within 48-72 hours until analysis. Samples will be treated and centrifuged by a single researcher under standardized conditions. The analysis will be performed in a laboratory in external quality assurance programs of the Spanish Society of Clinical Chemistry and Molecular Pathology.

**4.1.7. Lines 228-258: Remove 'it' and 'minute' from the beginning of sentences in this section. As above, remove the '+' here as well.**

Answer to reviewer:

Following the suggestions of the English language professional sought, we have replaced “it” for “this” in these sentences (Lines 206-220, Page 8):

Control breakfast (BF-1):
This will consist of 350 mL of water served at room temperature.

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

As the point 4.1.11.1 shows, the “Structure of intervention visits” section has been reworded and the other changes suggested by the reviewer have not been required.

**4.1.8. Line 231: water served at room temperature.**

Answer to reviewer:

We have introduced this modification in the “nutritional composition of each intervention arm” section (Lines 206-220, Page 8):

Control breakfast (BF-1):
This will consist of 350 mL of water served at room temperature.

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The

nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

**4.1.9. Line 231: Why was 350 mL of water provided for the control condition? How was this calculated? The foods provided for each breakfast condition will contain water, and ideally this should be balanced between all 3 conditions.**

Answer to reviewer:

The study by Ahuja et al [Ahuja KD et al: Am J Clin Nutr 2009, 90(2):298-303] provides abundant evidence of the effects of food intake on postprandial blood pressure and measures of arterial stiffness. In this work, “Participants were then provided with either 350 mL water (room temperature)”. The main results indicate that water intake did not elicit a significant change in brachial or central systolic pressure and the other variables, including aortic pressure and the augmentation index. The experimental group, besides water, took a light meal of 1301 KJ.
For these reasons, we decided to keep as a control group the intake of 350 mL of water served at room temperature, also including it in the rest of breakfasts, since their inclusion would not change the GI.

**4.1.10. Lines 232-240: Please provide the macronutrient content of these breakfasts. Remove the decimal place from the energy content of the breakfast foods. Consider reporting energy in kJ alongside/instead of kcal, as this is the SI unit for energy. Could the overall GI value of each breakfast also be stated? Could you also explain the reason for food choices? Are these food items that would typically be consumed at breakfast? Providing typical breakfast foods would improve the ecological validity of the study.**

**4.1.10.1. Lines 232-240: Please provide the macronutrient content of these breakfasts. Remove the decimal place from the energy content of the breakfast foods. Consider reporting energy in kJ alongside/instead of kcal, as this is the SI unit for energy. Could the overall GI value of each breakfast also be stated?**

Answer to reviewer:

The nutritional breakdown of the different breakfast is showed in the following table:

| Type of Breakfast | Glycaemic index, % | Energy, Kcal/KJ | Carbohydrates, % | Protein, % | Fat, % | Fibre, g |
|---|---|---|---|---|---|---|
| High Glycaemic index | 64.0 | 368 /1541 | 91.5 | 5.0 | 2.5 | 1.63 |
| Low Glycaemic index | 29.4 | 356 /1489 | 35.5 | 10.9 | 50.4 | 6.00 |

We have included the information about the macronutrient content and glycaemic index of these breakfasts in the methods section (Line 208-220, Page 8):

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

Additionally, we have introduced the information about the energy and glycaemic index in the abstract (methods) (Line 47, Page 2):

Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ).

**4.1.10.2. Could you also explain the reason for food choices? Are these food items that would typically be consumed at breakfast? Providing typical breakfast foods would improve the ecological validity of the study.**

Most of these products are consumed typically in the breakfast while others are not. However, the main objective of this study is to determine the effect of high GI breakfast vs. low GI in the variables studied. That has been the reason for the choice of these products and not others. However, as indicated by the reviewer, a possible and interesting work could be to evaluate the effect of typical breakfast of a region or area as may be the Mediterranean breakfast. But we could discuss it in a future study.

**4.1.11. Lines 241-258: What is a 'laboratory test sample'? If it is a blood sample, please state as such. The rationale for the time point's blood samples are being collected is sound, but additional blood samples, particularly between 0-60 min, would improve the interpretation of the glycaemic response to consumption of each breakfast.**

**4.1.11.1. Lines 241-258: What is a 'laboratory test sample'? If it is a blood sample, please state as such.**

Answer to reviewer:

In order to improve the understanding of this section, we have reworded it and now appears out of the intervention section as “Study procedures”, where we have used the term “blood sample” following the reviewer’s suggestion (Line 187, Page 7):

On arrival at the research unit, subjects will be weighed, and their height, waist circumference and hip circumference will be measured. Participants will be seated and remain in this position throughout the visit. After 5 minutes of rest, a peripheral blood pressure measurement will be performed. Immediately, the central blood pressure and hemodynamic parameters will be obtained. Next, cognitive performance will be assessed and fasting blood samples will be collected and central blood pressure and hemodynamic parameters will be determined again. Subjects will be provided with a randomly assigned breakfast to be consumed within 10 minutes. At the first bite, a timer will be started and additional measurements of central blood pressure and hemodynamic parameters will be taken every 15 minutes. Furthermore, another two cognitive performance evaluations and postprandial blood sampling will be completed at 60 and 120 minutes.

**4.1.11.2. The rationale for the time point's blood samples are being collected is sound, but additional blood samples, particularly between 0-60 min, would improve the interpretation of the glycaemic response to consumption of each breakfast.**

Answer to reviewer:

We are aware of additional blood samples would improve the interpretation of the glycaemic response to consumption of each breakfast. However, we have a limited funding and we consider the glycaemic response as an intermediate variable between vascular function and cognitive performance. Because of that, we have established these three time point's blood samples.

**5. Statistical analysis**

**5.1. It is stated that qualitative and quantitative data will be collected, but I am unsure which variables are qualitative. It might be useful to state the qualitative and quantitative variables, and how these will be handled statistically, separately.**

Answer to reviewer:

The main results will be measured from quantitative variables (i.e. central and peripheral blood pressure, augmentation index, central hemodynamic parameters, laboratory and cognitive performance variables, etc.) that will be analysed with the ANOVA test or its corresponding non-parametric test, correlation and regression. However, we will also use the corresponding tests for the analysis of the relationship between certain qualitative variables, such as gender, smoking habits and some clinical and demographic data. Besides this, some of the quantitative variables will be categorized, according to previously established cut points.

**5.2. It is stated on line 265 how data will be reported if normal or non-normally distributed, but how the data is handled if non-normally distributed is not stated in the statistical analysis section.**

Answer to reviewer:

We have changed the statistical analysis section, including some new paragraphs and reorganizing others, and now it reads as follows (Line 304, Page 11):

The normal distribution of variables will be verified using a Kolmogorov-Smirnov test. Quantitative variables will be displayed as the mean ± standard deviation if normally

distributed or as the median (interquartile range) if asymmetrically distributed. The qualitative variables will be expressed as frequencies. The data will be quantitated using the Q of the Cochrane test for qualitative data as well as repeated measures for ANOVA or the Friedman test if the data is non-normally distributed for quantitative data. To compare the differences among the three types of breakfast in quantitative variables, ANOVA test will be used, or Wilcoxon test if the data is non-normally distributed. We will use Least Significant Difference (LSD) test as post hoc analysis and the Chi Square test in qualitative variables. The relationship of quantitative variables to each other will be tested using Pearson's or Spearman's correlation as appropriate. The effect of the interventions can be modified by age, gender, cultural and socioeconomic level, body mass index, lifestyles and last menstruation date. To control the effect of such confounding factors on the study results and to evaluate adequately the effect of the interventions, a multivariate analysis will be performed using the General Lineal Model (GLM) over in basic or extended models. The contrasting hypothesis will establish an alpha risk of 0.05 because the limit of statistical significance. The data will be analysed using the IBM SPSS Statistics for Windows version 23.0 (Armonk, NY: IBM Corp).

**5.3. Main effects should also be assessed before post-hoc analyses (i.e. t-tests) are performed. In this case, repeated measures ANOVA may be an appropriate way of handling the quantitative data.**

Answer to reviewer:

We have modified the wording of the section referred to the main effects assessment, and now it reads as follows (Line 307, Page 12):

The data will be quantitated using the Q of the Cochrane test for qualitative data as well as repeated measures for ANOVA or the Friedman test if the data is non-normally distributed for quantitative data. To compare the differences among the three types of breakfast in quantitative variables, ANOVA test will be used, or Wilcoxon test if the data is non-normally distributed. We will use Least Significant Difference (LSD) test as post hoc analysis and the Chi Square test in qualitative variables.

**6. Discussion**

**6.1. Line 307: What is the EVIDENT study? I would urge you to avoid stating abbreviated study names in general.**

Answer to reviewer:

Following the suggestions of the third reviewer, this piece of evidence has been moved to the background section (Line 87, Page 4). However, we have included more information about the EVIDENT study:

In turn, the Lifestyles and Vascular Aging (EVIDENT) study [11] analysed the relationship between lifestyle and arterial aging in a sample of 1553 subjects who were free of cardiovascular disease. We concluded that low GI diets were associated with lower AIx values.

**6.2. Line 314-315: This appears to be the central aims of the study, but the response of insulin to breakfasts of different glycaemic indexes is not particularly novel. The rationale for this part of the study requires more detail.**

Answer to reviewer:

Our objective is really to analyse the relationship of vascular and cognitive function with postprandial glycaemic response for high versus low glycaemic index breakfasts. For this reason, we have modified the wording of the results expected (Line 339, Page 13):

In this project, we expect to demonstrate that low glycaemic index breakfast has a favourable effect on measures of vascular function and glycaemic response relative to high glycaemic index.

**6.3. Line 316: Is 'similarly' correct here? I'm not sure these two aims are similar. Consider changing to 'secondly'.**

Answer to reviewer:

Following the reviewer's suggestions, we have changed "similarly" to "secondly" (Line 342, Page 13):

Secondly, based on the findings of previous studies conducted on children and adolescents [26-30, 32], we hypothesize that we will see similar effects in healthy young adults.

**6.4. Lines 322-323: In this acute setting, I think the word 'improves' should be considered carefully. The acute effects of a given intervention are unlikely to improve plasma insulin values, for example. Therefore, I would suggest being more specific with this sentence, either explaining specifically the anticipated response to a given glycaemic breakfast, or perhaps use 'produces a favourable insulin response' in place of 'improve'.**

Answer to reviewer:

The indicated lines have been reworded for improving their clarity (Line 348, Page 13):

Therefore, according to our hypothesis, the results of the current study may explain the influence of glycaemic index on cognitive and vascular function. Moreover, given the high worldwide prevalence of cardiovascular diseases and its close relationship with cognitive decline, it would be interesting to know how the vascular parameters and cognitive processes that are affected by the type of breakfast consumed and as a function of lifestyle and dietary interventions.

**7. Table 1**

**7.1. I'm not sure what this table is supposed to show, and what the 'X' in the box means. If this simply states what will be done at each visit, I'm not sure it adds anything over what is stated in the text. What is the 'basal evaluation'? Basal is a specific term, meaning minimum requirement for sustaining life. For example, BMR is the amount of energy required to maintain basic celular processes to sustain life exclusively, and is not the same as RMR, which includes a 'resting' component to account for nonessential (albeit minimal) energy requirement processes. Therefore, I'm unsure what basal means in the context of this table.**

Answer to reviewer:

This table shows what will be done at baseline evaluation (which erroneously appears as basal evaluation) and at each intervention visit. As noted by the reviewer, it is not particularly informative, so we think that it can be removed.

**8. Figure**

**8.1. Replace 'o' for 'or', as stated at each visit.**

Answer to reviewer:

We have corrected this typographical error and we have replaced “basal evaluation” for “baseline evaluation”.

***Reviewer #3*** **Dr Enhad Chowdhury: Sanchez-Aguadero present a protocol paper describing the rationale and methods for a clinical trial examining the effect of breakfast GI upon a range of outcomes. While this study has the potential to contribute evidence in several areas, the description of, and rationale behind the study, requires greater clarity throughout.**

Answer to reviewer:

First, thank you for your work in reviewing this study protocol. Your contributions and suggestions will improve the understanding of the text.

**1. General comments:**

**1.1. Throughout the manuscript, the authors refer to the particular importance of breakfast due to its variable carbohydrate content-is there any evidence to suggest that the carbohydrate content of the breakfast meal is more variable than other eating occasions within populations? If not, I do not think that breakfast should be highlighted in this way, as any meal can be manipulated to the same extent for GI.**

Answer to reviewer:

As breakfast usually has an important load of carbohydrates, there can be variability in the glycaemic index of these carbohydrates. This was the idea that we wanted to express.

Nevertheless, as the background has been reworded to articulate more explicitly the novel aspect of the study, we have considered that this statement could be removed, and now the following sentence appears in its place (Line 101, Page 4):

Thus, breakfast would play a fundamental role because low and high GI meals eaten in the morning have resulted in better glycaemic control versus eating in the evening [19].

**1.2. At several points in the manuscript the authors refer to evidence "proving" certain effects. I would advise this phrasing could be removed in all cases and is particularly inappropriate when comparing opposing results from different studies.**

Answer to reviewer:

The phrasing mentioned has been removed in all cases.

**2. Specific comments**

**2.1. Abstract**

**2.1.1. Lines 32-34, the authors state "on the other hand, in terms of the influence of breakfasts with different glycaemic index on cognitive performance, different results have been obtained". It needs to be clarified whether the authors mean varying results have been obtained between studies (which I believe is the meaning) or that these results are different to others already stated. It needs to be clearer what the "different" refers to.**

Answer to reviewer:

“Different” refers to varying results obtained between studies. This has been clarified in the abstract (Line 38, Page 2):

Postprandial glycaemic response affects cognitive and vascular function. The acute effect of breakfast glycaemic index on vascular parameters is not sufficiently known. In turn, the influence of breakfasts with different glycaemic index on cognitive performance has been mostly studied in children and adolescents with varying results. Therefore, the purpose of this study is to analyse the postprandial effect of high and low glycaemic index breakfasts on vascular function and cognitive performance and their relationship with postprandial glycaemic response in healthy young adults.

**2.1.2. 40-42, if space allows then greater detail relating to the breakfast would be useful here, such as GI or energy content of breakfasts.**

Answer to reviewer:

Following the suggestions of the reviewer, we have introduced the information about the energy and glycaemic index of each breakfast in the abstract (methods) (Line 47, Page 2):

Each subject will complete three interventions with a washout period of one week: control breakfast (consisting of water); low glycaemic index breakfast (consisting of dark chocolate, walnuts, yogurt and an apple, with an overall glycaemic index of 29.4 and an energy contribution of 1489 KJ); and high glycaemic index breakfast (consisting of bread, grape juice and strawberry jam, with an overall glycaemic index of 64.0 and an energy contribution of 1541 KJ).

In addition, we have included the macronutrient content, energy and glycaemic index of each breakfast on the methods section, as the point 2.7.1 shows.

**2.1.3. Lines 52- authors state improvement in "school and work performance". As this study is in adults and there is extensive literature regarding the effect of breakfast consumption in children the reference to school should be removed here.**

Answer to reviewer:

Following the suggestions of the reviewer, the current wording of the discussion section in the abstract does not include the reference to school (Line 57, Page 3):

The differences in breakfast glycaemic index could affect vascular parameters, cognitive performance and postprandial glycaemic response with important applications and implications for general population. This provides necessary information for the establishment of new strategies in terms of nutritional education and work performance improvement.

**2.2. Background**

**2.2.1 Lines 63-68, the authors make several points relating to the distinction between GI and GL but this study only seeks to manipulate GI-is there a specific reason both need contrasting here?**

Answer to reviewer:

As the reviewer suggests, this study only seeks to manipulate GI and the distinction between GI and GL in the text is out of the context.

We have modified the text and now it reads as follows (Line 69, Page 3):

Diets with a high GI increase the risk of diseases related to chronic lifestyles such as type 2 diabetes mellitus [3, 4]. A recent meta-analysis of fourteen prospective studies found that high GI diets are associated with an increased risk of cardiovascular disease (CVD) [5] while a reduction in dietary GI can favourably affect the incidence of coronary disease in women [6].

**2.2.2. Lines 74-76, the authors finish these lines refer to their chosen measures with the statement "complement the information obtained with PWV". Can it be clarified if their chosen measures are ordinarily used as an addition to this gold standard measure or are used as a surrogate measure for arterial stiffness on their own? If this is the case, a reference should be provided.**

Answer to reviewer:

These parameters are measures of arterial stiffness on their own. This has been clarified and properly referenced in the text (Line 80, Page 3):

Likewise, the augmentation index (AIx) is a measure of wave reflection and arterial stiffness that has been shown to be a predictor of both future cardiovascular events and all-cause mortality [10].

**2.2.3. Line 79-80, the authors state food intake "causes a reduction in central hemodynamic parameters, Aix and PWV in healthy adults, particularly after 60 minutes"-the study referred to only obtained postprandial measures at 60 and 180 minutes. Therefore, I would advise the "particularly" here is inappropriate unless another reference can be provided to suggest the effect is less in the first 60 minutes.**

Answer to reviewer:

Following the reviewer's advice, we have modified the wording of this statement and now it reads as follows (Line 90, Page 4):

In this regard, a reduction in central hemodynamic parameters, AIx and PWV at 60 minutes from food intake has been reported in healthy adults perhaps because of an increase in insulin and/or visceral vasodilatation [12].

**2.2.4. Lines 80-84, there are several statements here where it is not clear where this information comes from e.g. "postprandial hypotension occurring in more than 70% of patients", "patients with higher BP values are those who experience it most". These statements should be clearly referenced.**

Answer to reviewer:

These statements come from the cohort study by Zanasi et al. [Zanasi A et al: J Hypertens 2012, 30(11):2125-2132], which assesses the prevalence and the prognostic power of postprandial hypotension in ambulatory hypertensive elderly patients without overt heart diseases. However, the background has been restructured to clarify the specific novel aspects of the current study, and we have thought that these sentences could be removed.

Thus, now the paragraph that included this information reads as follows (Line 92, Page 4):

Another possible explanation for these findings might be the postprandial hypotension that occurs after a meal due to decreased cortisol secretion and activation of parasympathetic system [13].

**2.2.5. Lines 84-85, "This variation in BP, and in particular that occurring after breakfast, it is an independent predictor of mortality" The referenced study did not assess mortality.**

Answer to reviewer:

This statement also comes from the cohort study by Zanasi et al. [Zanasi A et al: J Hypertens 2012, 30(11):2125-2132]. However, the background has been restructured to clarify the specific novel aspects of the current study, and we have thought that this sentence could be removed.

**2.2.6. Lines 87-92, I am not sure the specific comparisons of differing fatty acid compositions is particularly relevant for the current study.**

Answer to reviewer:

The specific comparisons of differing fatty acid compositions were included in the background in order to express that the impact of certain types of meals on vascular function has been tested.

As noted by the reviewer, this information is not particularly relevant for the current study, so we have removed it and now we only mention that a number of studies have been conducted for testing the effects of various macronutrients on vascular function (Line 95, Page 4):

For these reasons, although the effects of various macronutrients on vascular function have been explored in a number of studies [14-17], Taylor et al. [12] underlined the importance of analysing the impact of different types of meals on parasympathetic activity, CBP and vascular function parameters.

**2.2.7. Lines 93-95 are not clear. The study cited provided a fixed meal but this section gives the impression that differing CHO intake resulted in differing reductions in outcomes in healthy adults and postmenopausal women the study did not make a comparison between these groups and refers to the participants as "young and healthy".**

Answer to reviewer:

These findings had not been correctly referenced. They come from a study conducted by Greenfield et al. in postmenopausal women [Greenfield JR: Int J Cardiol. 2007 Jan 2;114(1):50-6].

We have introduced the correct reference in the text (Line 99, Page 4):

Of particular interest is the carbohydrate (CHO) content of a meal, which changes postprandial glucose and insulin levels and results in varying AIx reductions in postmenopausal women [18].

**2.2.8. Lines 102-104, after providing some contrary evidence the authors state that "the most important trial" is by Ahuja et al but don't provide a rationale for this. This is particularly important as the study by Ahuja did not compare GI but simply food vs no food, unlike the study cited in opposition which compared GI.**

Answer to reviewer:

We agree with the reviewer that the study by Ahuja et al should not be considered as the most important. For that reason, we have changed the wording of this paragraph (Line 103, Page 4):

However, despite the fact that breakfast patterns are associated with the metabolic profiles [20], few authors have studied its effect on cardiovascular responses. Ahuja et al. [21] found that a light breakfast (1301 KJ energy) reduced AIx, CBP and BP, and increased heart rate (HR) in adults versus fasting (water). In contrast, a trial aimed to compare the dietary effects of a high GI with a low GI breakfast replacement in obese and overweight individuals reported no differences in BP between breakfasts together with beneficial changes in fasting glucose and insulin levels unaffected [22].

**2.3. Objectives**

**2.3.1. Lines 122-124, is there a specific reason that the postprandial effect upon glucose and insulin is an objective here. The differences between these measures for high vs low GI meals is already very well established. This has been measured comprehensively in other studies. If it is the relationship between these measures and arterial stiffness that is the interesting comparison then this should be stated more clearly.**

Answer to reviewer:

As noted by the reviewer, with respect to the glycaemic control, our objective is really to analyse the relationship of vascular and cognitive function with postprandial glycaemic response for high versus low glycaemic index breakfasts.

Due to that, we have reworded the objectives in the abstract and methods, and now they read as follows:

(Line 42, Page 2): Therefore, the purpose of this study is to analyse the postprandial effect of high and low glycaemic index breakfasts on vascular function and cognitive performance and their relationship with postprandial glycaemic response in healthy young adults.

(Line 128, Page 5): The primary objective of this study is to evaluate the postprandial effect of low and high glycaemic index breakfasts on vascular function as measured by central blood pressure, augmentation index and pulse wave velocity in a sample of healthy young adults. The secondary aim is to assess the postprandial effect of low and high glycaemic index breakfasts on cognitive performance in a sample of healthy young adults. The third goal is to analyse the association between postprandial glycaemic response and vascular function and cognitive performance for high versus low glycaemic index breakfasts.

**2.4. Methods/Design**

**2.4.1. Line 132, I would advise referring to this as a control/fasting trial (as only water is being consumed) rather than a control breakfast as this might lead the**

**reader to think this will be a reference breakfast (i.e that this might be a "normal" breakfast rather than no breakfast).**

Answer to reviewer:

The study by Ahuja et al [Ahuja KD et al: Am J Clin Nutr 2009, 90(2):298-303] provides abundant evidence of the effects of food intake on postprandial blood pressure and measures of arterial stiffness. In this work, “Participants were then provided with either 350 mL water (room temperature)”. The main results indicate that water intake did not elicit a significant change in brachial or central systolic pressure and the other variables, including aortic pressure and the augmentation index. The experimental group, besides water, took a light meal of 1301 KJ.
For these reasons, we decided to keep as a control group the intake of 350 mL of water served at room temperature.

**2.4.2. Lines 144-145, needs rewording. It is also not clear by what is meant by "consecutive sampling will ensure comparability of the groups" as this is a crossover trial so there is only one group?**

Answer to reviewer:

We have rewording this line, which now reads as follows (Line 154, Page 6):

Because this is a crossover clinical trial, a consecutive sampling will ensure the comparability of interventions.

**2.4.3. Lines 154-155, is there a specific definition of what constitutes "excessive consumption of toxic substances" as an exclusion criteria?**

Answer to reviewer:

Any consumption of toxic substances will be excluded, so we have modified the wording of this exclusion criterion (Line 164, Page 6):

Subjects will be excluded with a history of cardiovascular events (acute myocardial infarction, stroke, etc.), hypertension, diabetes mellitus, dyslipidaemia, pharmacological treatment for any of these conditions, neurological and/or neuropsychological disease, or the consumption of toxic substances.

**2.5. Sample Size**

**2.5.1. Lines 160-162, "Considering the study design with three groups" is followed by "40 subjects would be required in each group to detect a minimum difference…..between two groups". Firstly there are no groups here but only several trials in one group and also the number of groups referred to changes. Are the authors confident they have conducted an appropriate calculation accounting for the repeated measures design-this needs clarification?**

Answer to reviewer:

Considering the design of paired data within each type of breakfast, and comparing them to the control condition, the sample size has been recalculated, by Granmo software (https://www.imim.es/ofertadeserveis/es_granmo.html), and the corresponding section reworded, as you can be seen at point 2.5.2.

**2.5.2. Line 162 "to detect a minimum difference of 7.5 points in Aix". What is the basis of this difference being relevant? Clinical relevance or based upon other research findings? Also a minimum difference of 7.5 points at a specific timepoint, or an incremental measure over the time course? This needs clarification.**

Answer to reviewer:

The CAFE study [Williams B et al: Circulation 2006, 113(9):1213-1225] found a reduction in the composite outcome of total cardiovascular events in the amlodipine regimen group, compared to the atenolol regimen group. There was a similar peripheral blood pressure decrease between treatment groups, but greater reductions in central blood pressure (4.3 (3.3, 5.4) mmHg) and central augmentation index (6.5 (5.8, 7.3) units) with the amlodipine regimen.

We have included this information in the sample size paragraph, and now it reads as follows (Line 179, Page 7):

The primary outcome variable is change in central augmentation index (AIx). The CAFE study [8] found a reduction in cardiovascular events associated with a decline of 6.5 (5.8, 7.3) points in the Aix. This is the basis of our calculation. The power calculation was a repeated measures design and compared both intervention breakfasts with a control breakfast with an alpha risk of 0.05 and a beta risk of 0.2. The SD was 10 with a correlation coefficient between the initial and final measurement of 0.7. Thus, 40 subjects are required to detect a minimum difference of 5 points in the AIx between two intervention breakfasts. A loss to follow-up of 5% was estimated.

**2.6. Variables and measurement instruments**

**2.6.1 Line 177, it is stated that regular diet will be assessed-is it to be standardised before trials (i.e the day before/morning of trials) as this may affect some of the metabolic parameters to be measured?**

Answer to reviewer:

The regular diet, together with other lifestyles, is going to be used as control variable on the study results for evaluating adequately the effect of the interventions.

**2.6.2. Lines 187-188, "The date of the last menstruation will be recorded due to the effect it may have on the study variables". Is this information going to be used in any way? Is date from menses going to first trial to be standardised between participants to attempt to control for any effects of time from menses.**

Answer to reviewer:

Although the stage of menstrual cycle should ideally be controlled, we have established a washout period of one week between each intervention for all the participants. Thus, the last menstruation date will be used as control variable on the study results for evaluating adequately the effect of the interventions.

**2.6.3. Line 208, papers using augmentation index e.g. ref 13 use an augmentation index normalised to HR, is this to be employed in this study? This should be clarified ideally with some rationale.**

Answer to reviewer:

We are going to employ an augmentation index corrected to a HR of 75 bpm. This has been clarified in the text (Line 274, Page 10):

The Mobil-O-Graph® device [38] will be used to estimate cardiac output and total peripheral vascular resistance and to measure central blood pressure, pulse wave velocity, reflection coefficient and augmentation index. This is affected by heart rate (HR) so its values will be corrected to a HR of 75 bpm. This device will be scheduled to perform continuous measurements at -10, 0, 15, 30, 45, 60, 75, 90, 105 and 120 minutes with the subject sitting and resting his arm on a rigid surface.

**2.6.4 Lines 213-215, there are numerous measures listed here to be obtained upon study entry but what is the purpose of obtaining these measures?**

Answer to reviewer:

These laboratory measures are going to be used as indicators of the inclusion criteria compliance and as control variables on the study results for evaluating adequately the effect of the interventions.

**2.6.5 Line 213-217, for variables to be measured from blood samples greater detail should be provided for collection and analysis methods-e.g. (venupuncture/fingerprick/cannula) as well as detailing analysis medium (whole blood/plasma/serum) and specific equipment and methods to be employed.**

Answer to reviewer:

We agree with the reviewer's assessment that the information provided in this section was insufficient, so we have detailed all the issues raised (Line 281, Page 11):

At the time of study entry and prior to the first intervention visit, fasting plasma creatinine, serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides values will be determined using standard enzymatic automated methods.
During each study visit, three cannula blood samples will be collected at -10, 60 and 120 minutes to measure serum glucose and insulin levels by ultraviolet-visible spectrophotometry and chemiluminescence, respectively. Serum will be isolated by centrifugation and stored at a – 20 ºC freezer within 48-72 hours until analysis. Samples will be treated and centrifuged by a single researcher under standardized conditions. The analysis will be performed in a laboratory in external quality assurance programs of the Spanish Society of Clinical Chemistry and Molecular Pathology.

**2.6.6. Lines 219-220, will the recall list of words be changed for each visit to prevent any carry over between visits? This should be specified.**

Answer to reviewer:

In effect, to prevent any carry over between visits, the recall list of words will be changed for each one of the three visits and for each evaluation within the same visit. This has been specified in the text (Line 292, Page 11):

For each one of the three visits and for each evaluation within the same visit, a list of 15 different words from the Rey Auditory Verbal Learning Test and its alternative versions [39-41] will be used to evaluate the immediate verbal memory via immediate recall over three attempts.

**2.6.7. Line 221, why is this period "approximately" 10 minutes? Is this likely to be substantially different between trials?**

Answer to reviewer:

Delayed verbal memory will be assessed at the end of the cognitive performance evaluation. After conducting a pilot study, we can confirm that this period will be exactly 10 minutes. Therefore, we have introduced this modification in the “cognitive performance evaluation variables” section (Line 295, Page 11):

Delayed verbal memory will be assessed by free recall of the words learnt in the first part of the evaluation after a period of 10 minutes.

**2.6.8 Lines 219-221, is this a commonly used test and if so is there a reference that can be provided to substantiate the efficacy of the test?**

Answer to reviewer:

In order to assess verbal memory, we have used standardized lists of 15 words from the Rey Auditory Verbal Learning Test and its alternative versions.

We have included this information in the text (Line 292, Page 11):

For each one of the three visits and for each evaluation within the same visit, a list of 15 different words from the Rey Auditory Verbal Learning Test and its alternative versions [39-41] will be used to evaluate the immediate verbal memory via immediate recall over three attempts.

We have also introduced the following references to support this task:

- Rey, A. (1964). L’Examen clinique en psychologie. Paris: Press Universitaire de France.
- Shapiro DM, Harrison DW. Alternate forms of the AVLT: a procedure and test of form equivalency. Arch Clin Neuropsychol. 1990;5(4):405-10.
- Lezak MD. Neuropsychological assessment. 2nd ed. New York: Oxford University Press; 1983.

**2.7. Intervention**

**2.7.1. Lines 232-240, as this study is specifically examining a comparison of GI at breakfast-the GI of the meals should be stated. Additionally the macronutrient composition of the breakfasts should be stated.**

Answer to reviewer:

The nutritional breakdown of the different breakfast is showed in the following table:

| Type of Breakfast | Glycaemic index, % | Energy, Kcal/KJ | Carbohydrate, % | Protein, % | Fat, % | Fibre, g |
|---|---|---|---|---|---|---|
| High Glycaemic index | 64.0 | 368 /1541 | 91.5 | 5.0 | 2.5 | 1.63 |
| Low Glycaemic index | 29.4 | 356 /1489 | 35.5 | 10.9 | 50.4 | 6.00 |

We have included the information about the macronutrient content and glycaemic index of these breakfasts in the methods section (Line 208-220, Page 8):

High glycaemic index breakfast (BF-2):
This will consist of 350 mL of water served at room temperature, 200 mL of grape juice (with 569 KJ/136 Kcal), 40 g of white bread (2 slices of 218 KJ/52 Kcal each) and 29 g of strawberry jam (with 536 KJ/128 Kcal) providing a total of 1541 KJ/368 Kcal. The nutrient composition of this meal was 91.5% carbohydrate, 2.5% fat, 5% protein and 1.63 g fibre, with an overall glycaemic index of 64.0.

Low glycaemic index breakfast (BF-3):
This will consist of 350 mL of water served at room temperature, a 150 g apple (with 339 KJ/81 Kcal), a 125 g low-fat natural yogurt (with 234 KJ/56 Kcal), 3 shelled walnuts (with 163 KJ/39 Kcal per unit) and 17.5 g of 72% dark chocolate (with 427 KJ/102 Kcal) supplying a total of 1489 KJ/356 Kcal. The nutrient composition of this meal was 35.5% carbohydrate, 50.4 % fat, 10.9% protein and 6 g fibre with an overall glycaemic index of 29.4.

Additionally, we have introduced the information about the energy and glycaemic index in the abstract (methods) as the point 2.1.2 shows.

**2.7.2. Line 241, I would recommend that this outlining of the study protocol is moved to earlier in the methods with the detailing of the specific measures afterwards. I would also recommend this might be easier to comprehend as a schematic rather than in written form as currently.**

Answer to reviewer:

As noted by the reviewer, we have moved this outlining of the study protocol earlier than the detailing of the specific measures (Line 188, Page 7).

In order to improve its understanding, we have also reworded it and now it appears out of the intervention section as “Study procedures”:

On arrival at the research unit, subjects will be weighed, and their height, waist circumference and hip circumference will be measured. Participants will be seated and remain in this position throughout the visit. After 5 minutes of rest, a peripheral blood pressure measurement will be performed. Immediately, the central blood pressure and hemodynamic parameters will be obtained. Next, cognitive performance will be assessed and fasting blood samples will be collected and central blood pressure and hemodynamic parameters will be determined again. Subjects will be provided with a randomly assigned breakfast to be consumed within 10 minutes. At the first bite, a timer will be started and additional measurements of central blood pressure and hemodynamic parameters will be taken every 15 minutes. Furthermore, another two cognitive performance evaluations and postprandial blood sampling will be completed at 60 and 120 minutes.

**2.7.3. Line 242, "Each of the three scheduled visits will last approximately 2 and a half hours", further detail is required here. What time are these visits to occur? What controls are in place for the participants beforehand?**

Answer to reviewer:

After performing a pilot study, we can confirm that each of the three scheduled visits will last 2 hours 40 minutes. In addition, these visits will occur between 8:15 am and 10:55 am and participants will be asked to fast for 12 hours overnight prior and to limit their physical activity, alcohol consumption and smoking during the previous 24-48 hours.

We have included this information in the methods section (Line 201, Page 8):

Each of the three scheduled visits will last 2 hours 40 minutes; this will occur between 8:15 am and 10:55 am. Participants will be asked to fast for 12 hours overnight prior and to limit their physical activity, alcohol consumption and smoking during the previous 24-48 hours.

**2.7.4 Line 244, what does "clinical" blood pressure mean here?**

Answer to reviewer:

It refers to the measurement of the peripheral blood pressure. In order to clarify this subheading, we have reworded it and now reads as follows (Line 265, Page 10):

Peripheral blood pressure.

**2.8. Statistical analysis**

**2.8.1. Lines 268-269, "student t-test for paired data" is to be used? Is this within groups over the timecourse or between groups at specific timepoints?**

Answer to reviewer:

Following the suggestions of the reviewer, we have modified the issues raised on the statistical analysis section, remaining as follows (Line 307, Page 12):

The data will be quantitated using the Q of the Cochrane test for qualitative data as well as repeated measures for ANOVA or the Friedman test if the data is non-normally distributed for quantitative data. To compare the differences among the three types of breakfast in quantitative variables, ANOVA test will be used, or Wilcoxon test if the data is non-normally distributed. We will use Least Significant Difference (LSD) test as post hoc analysis and the Chi Square test in qualitative variables. The relationship of quantitative variables to each other will be tested using Pearson's or Spearman's correlation as appropriate. The effect of the interventions can be modified by age, gender, cultural and socioeconomic level, body mass index, lifestyles and last menstruation date. To control the effect of such confounding factors on the study results and to evaluate adequately the effect of the interventions, a multivariate analysis will be performed using the General Lineal Model (GLM) over in basic or extended models. The contrasting hypothesis will establish an alpha risk of 0.05 because the limit of statistical significance. The data will be analysed using the IBM SPSS Statistics for Windows version 23.0 (Armonk, NY: IBM Corp).

**2.8.2. Lines 272-273. "the differences in the acute effect of each tested breakfast will be studied by gender" how is this to be done?**

Answer to reviewer:

The differences by sex in the response to each breakfast will be analysed. However, the main objective of our study is to determine the acute effect of high versus low GI breakfasts in the variables studied, so we think that this statement could be deleted, since it supposes additional information.

**2.8.3. Lines 273-277, "statements are made about the modifying effects of a range of variables upon the study outcomes here, but as this is a paired design this seems of limited importance? Additionally it is stated that the effects of such confounding factors will be controlled, but as there are so many of these variables surely the study is not adequately powered for this?**

Answer to reviewer:

To analyse the changes in the dependent variables due to each type of breakfast, we have considered that it would be appropriate to perform a multivariate analysis in two models. This has been described in the statistical analysis section (Line 316, Page 12):

To control the effect of such confounding factors on the study results and to evaluate adequately the effect of the interventions, a multivariate analysis will be performed using the General Lineal Model (GLM) over in basic or extended models.

**2.8.4. Will the order effect for trials be examined?**

Answer to reviewer:

As you can view at the design and setting section, the order of the three interventions will be determined by a randomization sequence. In this way, its effect for trials will be controlled.

**2.9. Methodological limitations**

**2.9.2. Lines 283-289, see above.**

Answer to reviewer:

As noted by the reviewer, these statements do not suppose a limitation in a paired design, so they could be removed.

**2.10. Ethical and safety considerations**

**2.10.1. I would recommend this section is moved to the front of the methods section.**

Answer to reviewer:

According to the reviewer's suggestions, we have moved the "ethical and safety considerations" section to the front of the methods (Line 137, Page 5).

**2.10.2. Line 299, "None of examinations poses" needs rephrasing.**

Answer to reviewer:

As noted by the reviewer above, we have reworded this sentence (Line 146, Page 6):

None of the testing could result in life-threatening risks for the subjects to be enrolled.

**2.10.3. Lines 302-303, "with the conditions foreseen" is unclear here. Rephrase.**

Answer to reviewer:

As the reviewer's suggests, we have rephrased it and now reads as follows (Line 149, Page 6):

Subject confidentiality will be ensured at all times in accordance with current laws and regulations on personal data protection (LOPD 15/1999 of 13 December) as well as with the conditions contemplated in Act 14/2007 on biomedical research.

**2.11. Discussion**

**2.11.1. Line 311, reference 37 seems like a particularly relevant piece of evidence and this should be referred to in the introduction.**

Answer to reviewer:

Following the reviewer's suggestions, this paragraph has been moved to the background section (Line 87, Page 4).

**2.11.2. Line 306, as in several other places "proving" is inappropriate here.**

Answer to reviewer:

As you can be seen below, this sentence has been reworded and "proving" does not already appear (Line 328, Page 12).

**2.11.3. Lines 312-313, "few authors have proven the effect of breakfast on vascular function and hemodynamic parameters 19,20". This wording is unclear, what have these authors actually found?**

Answer to reviewer:

As it is detailed on the background section, contrasting results have been reported by Ahuja and Pal [Ahuja KD et al: The American journal of clinical nutrition 2009, 90(2):298-303; Pal S, et al: J Am Coll Nutr 2008, 27(3):387-393]. This specification has been introduced in the mentioned line (Line 338, Page 13):

Contrasting results have been reported about the effect of breakfast on vascular function and hemodynamic parameters [21, 22].

**2.11.4. Line 314, reduced should be reduce. "Insulin blood levels" should be rephrased.**

Answer to reviewer:

As the objectives of the study have been reconsidered, we have modified the wording of the results expected and the term “insulin blood levels” has been replaced for “glycaemic response” (Line 339, Page 13):

In this project, we expect to demonstrate that low glycaemic index breakfast has a favourable effect on measures of vascular function and glycaemic response relative to high glycaemic index.

**2.11.5. Line 315, as insulin will be increased relative to control in the high and low GI trials, this line should clearly indicate that insulin will be lower than high GI, not lower than baseline e.g. reduce blood pressure and insulin concentrations relative to the high GI condition.**

Answer to reviewer:

As the reviewer suggests, we have reworded this line (Line 339, Page 13):

In this project, we expect to demonstrate that low glycaemic index breakfast has a favourable effect on measures of vascular function and glycaemic response relative to high glycaemic index.

**2.11.6. Lines 317-321, are these predictions based upon evidence comparing effect of GI from previous studies (e.g. several studies by Lamport et al in 2013/2014) or just predictions?**

Answer to reviewer:

Based on the results found on previous studies performed on children and adolescents, we hypothesize that similar effects on different cognitive functions could be found in young healthy adults, since their cognitive processes are healthy and not impaired as in diabetic or obese patients. Results to be obtained in this study may help us in understanding how GI can influence on cognitive processes. This information has been stated in the text, and the current paragraph reads as follows (Line 342, Page 13):

Secondly, based on the findings of previous studies conducted on children and adolescents [26-30, 32], we hypothesize that we will see similar effects in healthy young adults. Thus, we expect that the low glycaemic index breakfast has a positive impact on immediate and delayed verbal memory and verbal fluency, while high glycaemic index breakfast will positively affect attention, processing speed and working memory.

**2.11.7. Lines 322-325 are not clear and should be reworded.**

Answer to reviewer:

The indicated lines have been reworded for improving their clarity (Line 348, Page 13):

Therefore, according to our hypothesis, the results of the current study may explain the influence of glycaemic index on cognitive and vascular function. Moreover, given the high worldwide prevalence of cardiovascular diseases and its close relationship with cognitive decline, it would be interesting to know how the vascular parameters and cognitive processes are affected by the type of breakfast consumed in regards to the design of novel lifestyle and dietary interventions.

**2.11.8. Lines 325-328, the authors have already stated in lines 318-321 that they expect different positive effects upon different aspects of cognitive performance by the high and low GI breakfasts. Therefore in these final lines they refer to "a breakfast that improves cognitive performance" which type of breakfast is this expected to be? Or is this advocating simply consumption of breakfast as opposed to fasting?**

Answer to reviewer:

This sentence does not refer to a specific breakfast. Our idea was to state that each breakfast could improve certain cognitive functions so it would be possible to adapt its composition for increasing performance at each type of job.

In order to clarify it, we have reworded this paragraph (Line 354, Page 13):

Finally, our results may provide tools for adapting breakfast composition to the tasks that should be performed and improve work performance.

**2.11.9. Line 328, the reference to school here should be removed.**

Answer to reviewer:

As noted by the reviewer, the reference to school at the end of the discussion has been removed (Line 354, Page 13):

Finally, our results may provide tools for adapting breakfast composition to the tasks that should be performed and improve work performance.

**2.12. Table 1 and Figure 1 don't seem particularly informative so I would suggest can be removed.**

Answer to reviewer:

We agree with the reviewer that the Table 1 does not add anything over what is stated in the text, so it could be removed. However, we think that the Figure 1 should not be deleted, since it schematically shows the phases of this crossover clinical trial, improving its understanding.

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