Journal of Clinical and Translational Research
Peer review process file 08.202206.011

# The action of phytochemicals present in cocoa in the prevention of vascular dysfunction and atherosclerosis

Thayzis de Paula Silva*, Aline Andressa Silva, Mayla Cardoso Fernandes Toffolo, Aline Silva de Aguiar

*Corresponding author
Thayzis de Paula Silva
*School of Nutrition, Federal University of Ouro Preto, Campus Morro do Cruzeiro, Ouro Preto, Minas Gerais, Brazil.*

Handling editor:
Michal Heger
*Department of Pharmaceutics, Utrecht University, the Netherlands*
*Department of Pharmaceutics, Jiaxing University Medical College, Zhejiang, China*

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1st Editorial decision
09-May-2022

Ref.: Ms. No. JCTRes-D-22-00022
Effects of cocoa and dark chocolate phytochemicals on the prevention of atherosclerosis
Journal of Clinical and Translational Research

Dear Master Silva,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below and attached to this decision letter.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jun 08, 2022.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: This review is based on a wrong conclusion
The statement „The consumption of chocolate with a high concentration of cocoa, greater than 83%, proved to be an important preventive strategy" is incorrect. See my attachment. Th cocoa percentage of a chocolate does not necessarily represent its content on active principle (see Table 4). Sample DC 6, for example, a chocolate with 99% cocoa contains 900 mg of total flavanols in 500 g chocolate compared to a 100% chocolate that contained the 900 mg of total flavanols in 115 g of chocolate. It is very much dependent on how a chocolate is processed. Company Lindt, for example, has a patent on its processing method which makes the chocolate soft and nice to eat. The chocolate puree is stirred for 3 days during various high temperatures. Oxygen and high temperature is, thus, destroying the active principle.
The authors should focus on the amount of active principle in dark chocolate rather than on cocoa percentages. The Kuna Indians in Panama and Columbia, who consume daily 600 to 900mg of flavanols (as well as other polyphenols), have a very low risk of cardiovascular disease. The group of Schroeter has recalculated from urinary excretion of flavanol metabolites that a dose around 900 mg flavanols is what they consume. Once the inhabitants leave their island and live a Western life style in the US or somewhere else, they have the same high cardiovascular risk as the Western population.
So to conclude that >83% dark chocolate is a preventive strategy is misleading.

Reviewer #2: JCTRes-D-22-00022

This paper reviews the effects of cocoa on atherosclerosis.
It is quite comprehensive and sound, with the notable exception of free radical removal by (poly)phenols. This is last-century science, flavonoids do not remove free radicals and ROS because they are poorly bioavailable and poorly reactive. See papers by the experts, e.g. Henry Forman or Helmut Sies and REMOVE the whole section.
The effects on platelets is missing and is important. See and discuss what this reviewer found to be the first paper on chocolate and health PMID: 19234942
Chocolate is very energy-dense and should be consumed with caution, hence a warning statement must be included toward the end. Otherwise the whole paper appears as promoting chocolate as a medicine. It is not.
Another disclaimer to add is that the near totality of the reviewed studies has been done in vitro, with supra-physiological concentrations. Whether the same effects are seen in vivo is still to be validated.
Finally, the paper is well-written, but some sentences are convoluted and the authors should edit it by using a professional editor or Grammarly or Writefull or something.

Reviewer #3: Review of manuscript ref. antioxidants-1641870
Title: "Effects of cocoa and dark chocolate phytochemicals on the prevention of atherosclerosis"
Authors: anonimous

General comment: the study reviews the recent data related to the beneficial effect of intake of cocoa and dark chocolate on the prevention of vascular dysfunction and atherosclerosis. The number of studies in the recent years on this topic is constantly increasing and it is necessary to update regularly the information; thus, this sort of short reviews supporting the FDA and EFSA claims on cocoa flavonoids and cardiovascular health should always be welcome. Although there are a number of recent references missing, perhaps the goal of the authors was to point out some particular aspects instead of a throughout revision of all available data; in that case, the authors should clearly state in introduction those specific items/markers/parameters that they would like to point out. Some specific comments are detailed below:

Specific comments:
1) Pages should be numbered.
2) Introduction, second paragraph, line 2; it should be 4 as super index.
3) Methodology, epigraph phytochemicals in cocoa, line 5; the statement is incorrect, flavonoids is the generic name for all compounds included in the noted subfamilies, thus, flavan-3-ols or flavanols are just a subfamily and not all flavonoids are also called flavan-3-ols.
4) Line 4 before epigraph: Effects of cocoa phytochemicals on atherosclerosis; greek letter kappa should be instead of K within NF-kB. The same applies to the rest of the text.
5) Epigraph: Effects of cocoa phytochemicals on atherosclerosis, paragraph starting with Martins et al. (2020); it should be noted that in this article the chemo-protective effect was reported for the cocoa phenolic extract and epicatechin, instead of catechin.
6) Three lines before the table; it says that table 1 contains the studies whose intervention was the consumption of chocolate or cocoa, in relation to antioxidant and anti-inflammatory metabolic outcomes, but it also contains results related to lipid profile, blood pressure and blood glucose. Perhaps it should be better just to say that the main outcomes are summarized.
7) Table 1; size and font of text within the table is inconstant, it should be uniform.
8) Table 1; reference by Cavarretta et al. should be number 45, instead of 44.

Reviewer #4: Further analysis is still needed to draw conclusion made in your manuscript. Suggest to do a proper scoping or systematic review using critical appraisal tools as a guidance/ checklist to produce a strong paper.

Reviewer #5: The theme of the present manuscript is relevant. However, in my opinion, it needs to be improved to be published. There are many typos and the English language should be revised. There are also some scientific errors. Please find below the corresponding details:
- The pages are not numbered.
- In the abstract, on line 48: metabolic should be removed

- Page 2
Line 6: remove the. before CNDS
Line20: 4 should be a superscript
Line 43: remove the at the beginning of the sentence
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Line 23: please remove conference and rewrite the sentence
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Line 0: the following sentence is not correct: beneficial effects to the organism is unlikely. There are more recent papers that show that cocoa colonic metabolites are responsible for cocoa's bioactive properties. One of these papers is Sarria et al 2020, among others.
Line 20: please check this figure: 2,000 mg/d
Line 23: 2,000 mg/d is well apart from 105 mg/day
Line 38: change ON to NO
- Page6
Line 12: Do you mean 4 mg/day, please check this! You are taking about a regular intake of a cocoa product, this should be stated!
Line 14: IL-10 is antiinflammatory
Line 15: What other molecules?
Line 23: The, should not be there
Line 40: remove .
- Page 12
Line 11: at least a range could be established according to the literature cited
Conclusion: It should be stated that cocoa is a high-energy food and thus the amount ingested should be considered with caution. It is not right to say the more the better. Also, the idea that cocoa should be consumed within a healthy diet is missing and should be included. In general, the conclusion is too broad and not informative enough.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

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Authors' response

Juiz de Fora, June 08, 2022.

Dear. Michal Heger

Editor-in-Chief of Journal of Clinical and Translational Research

**Response letter to the editor**

We submit the article entitled "The Action of phytochemicals present in cocoa in the prevention of vascular dysfunction and atherosclerosis" produced by Thayzis de Paula Silva, Aline Andressa Silva, Mayla Cardoso Fernandes Toffolo, and Aline Silva de Aguiar. We would like

to inform you that in this period of review and correction of the manuscript, an author who participated in this review process in this second phase was included. The author is Mayla Cardoso Fernandes Toffolo, PHd. From the Federal University of Ouro Preto (email:mayla.toffolo@ufop.edu.br). The authors have no conflicts of interest to declare and confirm that each one has made substantial contributions to the information or materials submitted for publication. All authors have read and approved the final manuscript, and being such, agree to publish the data in your journal, in the order in which their names are listed.

Changes suggested by reviewers are marked in red in the text allowing reviewers to quickly verify all changes made.

Reviewer #1: This review is based on a wrong conclusion
The statement, The consumption of chocolate with a high concentration of cocoa, greater than 83%, proved to be an important preventive strategy" is incorrect. See my attachment. The cocoa percentage of chocolate does not necessarily represent its content on active principle (see Table 4). Sample DC 6, for example, chocolate with 99% cocoa contains 900 mg of total flavonols in 500 g of chocolate compared to a 100% chocolate that contained the 900 mg of total flavonols in 115 g of chocolate. It is very much dependent on how chocolate is processed. Company Lindt, for example, has a patent on its processing method which makes the chocolate soft and nice to eat. The chocolate puree is stirred for 3 days during various high temperatures. Oxygen and high temperature are, thus, destroying the active principle.
The authors should focus on the amount of active principle in dark chocolate rather than on cocoa percentages. The Kuna Indians in Panama and Columbia, who consume daily 600 to 900mg of flavanols (as well as other polyphenols), have a very low risk of cardiovascular disease. The group of Schroeter has recalculated from urinary excretion of flavanol metabolites that a dose of around 900 mg flavanols is what they consume. Once the inhabitants leave their island and live a Western lifestyle in the US or somewhere else, they have the same high cardiovascular risk as the Western population. So to conclude that >83% dark chocolate is a preventive strategy is misleading.

**R: Suggestion accepted, conclusion has been rewritten in the text.**

**The conversion of cocoa % concentration to milligrams of flavonoids was discussed as a limitation of the study highlighted in red in the text.**

Reviewer #2: This paper reviews the effects of cocoa on atherosclerosis.

It is quite comprehensive and sound, with the notable exception of free radical removal by (poly)phenols. This is last-century science, flavonoids do not remove free radicals and ROS because they are poorly bioavailable and poorly reactive. See papers by the experts, e.g. Henry Forman or Helmut Sies, and REMOVE the whole section.

The effects on platelets are missing and are important. See and discuss what this reviewer found to be the first paper on chocolate and health PMID: 19234942

Chocolate is very energy-dense and should be consumed with caution, hence a warning statement must be included toward the end. Otherwise, the whole paper appears as promoting chocolate as a medicine. It is not.

Another disclaimer to add is that the near totality of the reviewed studies has been done in vitro, with supra-physiological concentrations. Whether the same effects are seen in vivo is still to be validated.

Finally, the paper is well-written, but some sentences are convoluted and the authors should edit it by using a professional editor or Grammarly or Write full or something.

**R: Suggestion accepted, session removed.**

**Warning added at the end of the thread marked in red.**

**Suggestion accepted, was discriminated in the table in vitro study and humans.**

**Grammar revised.**

Reviewer #3: Review of manuscript ref. antioxidants-1641870
Title: "Effects of cocoa and dark chocolate phytochemicals on the prevention of atherosclerosis"
General comment: the study reviews the recent data related to the beneficial effect of intake of cocoa and dark chocolate on the prevention of vascular dysfunction and atherosclerosis. The number of studies in recent years on this topic is constantly increasing and it is necessary to update regularly the information; thus, this sort of short

review supporting the FDA and EFSA claims on cocoa flavonoids and cardiovascular health should always be welcome. Although several recent references are missing, perhaps the goal of the authors was to point out some particular aspects instead of a throughout revision of all available data; in that case, the authors should clearly state in the introduction those specific

items/markers/parameters that they would like to point out. Some specific comments are detailed below:

A new search was carried out for studies that complemented the articles already included in this review, however, no study of great relevance was found to be included in this work, according to the established criteria. Specific comments:
1) Pages should be numbered. **Suggestion accepted.**

2) Introduction, second paragraph, line 2; it should be 4 as a super index. **Suggestion accepted.**

3) Methodology, epigraph phytochemicals in cocoa, line 5; the statement is incorrect, flavonoids is the generic name for all compounds included in the noted subfamilies, thus, flavan-3-ols or flavanols are just a subfamily and not all flavonoids are also called flavan-3-ols. **Corrected statement.**

4) Line 4 before epigraph: Effects of cocoa phytochemicals on atherosclerosis; greek letter kappa should be instead of K within NF-kB. The same applies to the rest of the text. **Suggestion accepted**.

5) Epigraph**: Effects of cocoa phytochemicals on atherosclerosis, paragraph starting with Martins et al. (2020); it should be noted that in this article the chemo-protective effect was reported for the cocoa phenolic extract and epicatechin, instead of catechin. It was suggested by the second reviewer to remove this reference which was included in the section "antioxidant activity"**

6) Three lines before the table; it says that table 1 contains the studies whose intervention was the consumption of chocolate or cocoa, about antioxidant and anti-inflammatory metabolic outcomes, but it also contains results related to lipid profile, blood pressure, and blood glucose. Perhaps it should be better just to say that the main outcomes are summarized. **Suggestion accepted**.

7) Table 1; size and font of text within the table are inconstant, it should be uniform. **Suggestion accepted.**

8) Table 1; reference by Cavarretta et al. should be number 45, instead of 44. Suggestion accepted.

Reviewer #4: Further analysis is still needed to draw the conclusion made in your manuscript. Suggest doing a proper scoping or systematic review using critical appraisal tools as a guidance/ checklist to produce a strong paper.

**R: The analyzes were performed according to the study proposal, which is a narrative type review. Thus, all adjustments are being made to improve the work according to the methodology of a narrative review. The suggestions made by the fourth reviewer described in the pdf are marked in red in the text.**

Reviewer #5: The theme of the present manuscript is relevant. However, in my opinion, it needs to be improved to be published. There are many typos and the English language should be revised. There are also some scientific errors. Please find below the corresponding details: **Grammar revised.**

- The pages are not numbered. **Suggestion accepted.**

- In the abstract, on line 48: metabolic should be removed. **Suggestion accepted**.

Page 2

Line 6: remove the. before CNDS. **Suggestion accepted.**

Line20: 4 should be a superscript. **Suggestion accepted.**

Line 43: remove the at the beginning of the sentence. **Suggestion accepted.**

Line 58: please edit the sentence as follows: there are still inconsistencies regarding... **Suggestion accepted.**

Page 3

Line 18: the following sentence does not make sense: focusing on the included and excluded theme**. Suggestions accepted and rewritten.**

Line 23: please remove the conference and rewrite the sentence. **Suggestions accepted and rewritten.**

Page 5

Line 0: the following sentence is not correct: beneficial effects to the organism are unlikely. There are more recent papers that show that cocoa colonic metabolites are responsible for

cocoa's bioactive properties. One of these papers is Sarria et al 2020, among others. **Suggestions accepted and rewritten.**

Line 20: please check this figure: 2000 mg/d. **Verified.**

Line 23: 2000 mg/d is well apart from 105 mg/day. Because the study carried out by Ottaviani et al. (2015) evaluated the safety and efficacy of consuming cocoa flavanols in healthy adults and concluded that ingestion in amounts of up to 2000 mg/d in 12 weeks had no adverse effects on the health of men and women.

Line 38: change ON to NO**. Verified.**

Page 6

Line 12: Do you mean 4 mg/day, please check this! You are talking about a regular intake of a cocoa product, this should be stated! **Verified.**

Line 14: IL-10 is anti-inflammatory. **Verified.**

Line 15: What other molecules? It was not cited in the article

Line 23: The, should not be there. **Verified and withdrawn.**

Line 40: remove. **Verified and withdrawn**.

Page 12

Line 11: at least a range could be established according to the literature cited. Verified. Conclusion: It should be stated that cocoa is a high-energy food and thus the amount ingested should be considered with caution. It is not right to say the more the better. Also, the idea that cocoa should be consumed within a healthy diet is missing and should be included. In general, the conclusion is too broad and not informative enough. **Suggestions accepted and rewritten.**

Please find enclosed the revised version of the manuscript. We hope that all changes requested were made. We look forward to the final decision of the editors and thank you for your attention.

Sincerely yours,

Thayzis de Paula Silva

Corresponding author

thayzis_jf6@hotmail.com

2nd Editorial decision
22-Jun-2022

Ref.: Ms. No. JCTRes-D-22-00022R1
The action of phytochemicals present in cocoa in the prevention of vascular dysfunction and atherosclerosis
Journal of Clinical and Translational Research

Dear Master Silva,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jul 22, 2022.

To submit a revision, go to https://www.editorialmanager.com/jctres/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

EDITOR:
- There are still grammatical errors in the paper. Please engage a native speaker or a third party language editing service to streamline the manuscript. If none of these are available to you, please contact the editor (m.heger@jctres.com). We can assign an in-house editor who will perform a deep dive and language polishing for a fee.
- Please pay particular attention and implement the essence of Reviewer 2's remark regarding the in vitro-in vivo dilemma. It is a very good point that should not be undervalued.

Reviewer #2: This paper reviews the effects of cocoa on atherosclerosis.
It is quite comprehensive and sound, with the notable exception of free radical removal by

(poly)phenols. This is last-century science, flavonoids do not remove free radicals and ROS because they are poorly bioavailable and poorly reactive.
See papers by the experts, e.g. Henry Forman or Helmut Sies and REMOVE the whole section.
The effects on platelets is missing and is important. See and discuss what this reviewer found to be the first paper on chocolate and health PMID: 19234942
Chocolate is very energy-dense and should be consumed with caution, hence a warning statement must be included toward the end. Otherwise the whole paper appears as promoting chocolate as a medicine. It is not.
Another disclaimer to add is that the near totality of the reviewed studies has been done in vitro, with supra-physiological concentrations. Whether the same effects are seen in vivo is still to be validated.
Finally, the paper is well-written, but some sentences are convoluted and the authors should edit it by using a professional editor or Grammarly or Writefull or something.

Reviewer #3: All my comments and queries have been conveniently addressed; thus, I recommend to accept the revised version 1 for publication at JCTR.

Reviewer #4: Please refer to more published review papers on how to do a proper data extraction table. There is too much information slotted in the 'Intervention' column which is confusing.

The conclusion stated is just compiling all the parameters from the findings. There is no in-depth analysis from data extracted which can draw a stronger conclusion.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

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Authors' response

Dear. Michal Heger

Editor-in-Chief of Journal of Clinical and Translational Research

**Response letter to the editor**

We submit the article entitled "The Action of phytochemicals present in cocoa in the prevention of vascular dysfunction and atherosclerosis" produced by Thayzis de Paula Silva, Aline Andressa Silva, Mayla Cardoso Fernandes Toffolo, and Aline Silva de Aguiar. We would like to inform you that in this period of review and correction of the manuscript, an author who participated in this review process in this second phase was included. The author is Mayla Cardoso Fernandes Toffolo, PHd. From the Federal University of Ouro Preto (email:mayla.toffolo@ufop.edu.br). The authors have no conflicts of interest to declare and

confirm that each one has made substantial contributions to the information or materials submitted for publication. All authors have read and approved the final manuscript, and being such, agree to publish the data in your journal, in the order in which their names are listed.

Changes suggested by reviewers are marked in red in the text allowing reviewers to quickly verify all changes made.

Reviewer #2: This paper reviews the effects of cocoa on atherosclerosis.

It is quite comprehensive and sound, with the notable exception of free radical removal by (poly)phenols. This is last-century science, flavonoids do not remove free radicals and ROS because they are poorly bioavailable and poorly reactive. See papers by the experts, e.g. Henry Forman or Helmut Sies and REMOVE the whole section.

**Session has already been removed**.

The effects on platelets is missing and is important. See and discuss what this reviewer found to be the first paper on chocolate and health PMID: 19234942

**This content is described in the second paragraph of the third session.**

Chocolate is very energy-dense and should be consumed with caution, hence a warning statement must be included toward the end. Otherwise the whole paper appears as promoting chocolate as a medicine. It is not.

**The warning statement was included at the end of the text.**

Another disclaimer to add is that the near totality of the reviewed studies has been done in vitro, with supra-physiological concentrations. Whether the same effects are seen in vivo is still to be validated.

**According to the inconsistency of the in vitro studies addressed in this review, both were withdrawn because they did not present safe results when extrapolated to humans. Only one study cited in the table whose author Nanetti and collaborators was kept where part of the research was carried out in vitro and in vivo.**

Finally, the paper is well-written, but some sentences are convoluted and the authors should edit it by using a professional editor or Grammarly or Writefull or something.

**Revised certification article attached.**

Reviewer #4: Please refer to more published review papers on how to do a proper data extraction table. There is too much information slotted in the 'Intervention' column which is confusing.

**The "intervention" column has been rewritten with key information.**

The conclusion stated is just compiling all the parameters from the findings. There is no in-depth analysis from data extracted which can draw a stronger conclusion**.**

**Rewritten conclusion.**

**The other comments sent in the attachment were adjusted directly in the text.**

Please find enclosed the revised version of the manuscript. We hope that all changes requested were made. We look forward to the final decision of the editors and thank you for your attention.

Sincerely yours,

Thayzis de Paula Silva
Corresponding author

thayzis_jf6@hotmail.com

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3rd Editorial decision
27-Jul-2022

Ref.: Ms. No. JCTRes-D-22-00022R2
The action of phytochemicals present in cocoa in the prevention of vascular dysfunction and atherosclerosis
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Journal of Clinical and Translational Research
Peer review process file 08.202206.011

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers: