PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Design of a randomized controlled trial of the effects of empagliflozin on myocardial perfusion, function and metabolism in type 2 diabetes patients at high cardiovascular risk (The SIMPLE trial)
AUTHORS	Jürgens, Mikkel; Schou, Morten; Hasbak, Philip; kjær, andreas; Wolsk, Emil; Zerahn, Bo; Wiberg, Mikkel; Brandt, Niels; Gæde, Peter; Rossing, Peter; Faber, J; Inzucchi, Silvio; Gustafsson, Finn; Kistorp, Caroline

VERSION 1 – REVIEW

REVIEWER	Md. Shahriar Mahbub Research Fellow, Bangladesh University of Health Sciences, Bangladesh
REVIEW RETURNED	02-Mar-2019

GENERAL COMMENTS	This is a well-designed study which aims to further explore the effects of empaglifozin in T2D patients. This is expected to add to the body of knowledge on this important aspect of this specific drug. I have the following suggestions/queries: 1. A schema would be helpful to better understand the sample with both primary and secondary end-points. 2. The authors can take this recent article into consideration for literature review: https://doi.org/10.1007/s40265-018-0937-z 3. The recommended starting dose is 10 mg. What is the rationale behind selecting 25 mg? 4. Is the duration of the intervention (13 weeks) arbitrary or following a specific guideline? 5. Is there any termination criteria (e.g. safety grounds)? The authors have done well and I wish them success.

REVIEWER	Andrea Baragetti University of Milan - Department of Pharmacological and Biomolecular Sciences
REVIEW RETURNED	03-Mar-2019

GENERAL COMMENTS	1) The methodology of adipose tissue determination of cytokines for mRNA is not properly presented. How tissue will be extracted and how it will be processed for the gene expression analysis profile. Moreover, why did not authors consider ELISA determination on circulating plasma? Do they think this would not be less invasive and additive in terms of scientific knowledge?

	2) Oral glucose tolerance test. Why did authors not include glucagon determination? 3) Did authors consider lactate and troponin-I determination as well? Would it be rationale? 4) Urine and blood sample determination should be better clarified in the methods. Which are the urine metabolite considered for renal evaluation (beside the instrumental analysis)? 5) Please, change “pharmaceutical dosage” into “pharmacological dosage”. For placebo: do authors consider similar ingredients for palatability? 6) Why did authors consider only the 25 mg form? Although the rationale is to estimate maximal empagliflozin on the outcome, would it be more rationale to consider a dose-escalating approach in such a particular study? 7) Why did authors not consider a cross-over approach? 8) I suggest to implement the time-line design of the study graphically. 9) Pag.6 line 21: why did authors consider adenosine rather than nitro-glycerin? Why did authors consider such dosage i.v.? 10) Better definition of echocardiography procedure is suggested. 11) Diabetes disease vintage is roughly considered and explained in table 1. Authors report that patients with diabetes diagnosed at least three months prior to randomization will be included. However, which is the maximal disease duration conceived. It is pivotal to report this point as this will be crucial for outcomes. Do authors believe to find more important effects on ling-lasting diabetes vs early-onset diabetes? 12) Do authors conceive to measure C-reactive protein as well? What do authors think such potent pharmacological compound, empagliflozin, would add on top of recently proposed anti-inflammatory therapies in high-risk patients (see: Ridker PM JACC 2018; Verma S JACC 2018). Please, comment in light of previous point as well. 13) In table 1: “stable dose of anti-diabetic therapy” is elusive: could authors better explain?

REVIEWER	Tarissa Beatrice Zanata Petry Hospital Alemão Oswaldo Cruz - Brazil
REVIEW RETURNED	20-May-2019

GENERAL COMMENTS	This is a randomized, double blind controlled trial, Please correct the name "DECLAIRE TIMI trials" to DECLARE TIMI trials. What is "MBF" in the second paragraph of the session Statistical Analysis, Sub-study key endpoint? Inclusion Criteria: Hba1c of ≥ 48 mmol/L and ≤ 86 mmol/L at screening for patients on background therapy or Hba1c of ≥ 48 mmol/L and ≤ 75 mmol/L at screening for drug-naïve patients. Why? There are a lot of initials without its description in table 1. Exclusion Criteria: History of ischemic or hemorrhagic stroke > 2 months prior to informed consent - what about vascular malformation as a cause of hemorrhagic stroke? Your flowchart of study "visits" (table 2) is not consistent with your text ("Trial visits and procedures - Visits"). Please review and rewrite it.

VERSION 1 – AUTHOR RESPONSE

Response to reviewers

Reviewer 1

1. A schema would be helpful to better understand the sample with both primary and secondary end-points.

We agree this would be helpful, and we hope that the revised table 2 (p. 16) is sufficient.

2. The authors can take this recent article into consideration for literature review:
https://doi.org/10.1007/s40265-018-0937-z

We thank the reviewer for bringing this paper to our attention. We have added the papers https://doi.org/10.2337/dc16-0041, and https://doi.org/10.1093/eurheartj/ehv728 from the reference list.

3.The recommended starting dose is 10 mg. What is the rationale behind selecting 25 mg?

We chose the high dose of 25 mg, since the current trial examines the metabolic effects of empagliflozin in secondary endpoints, and previous studies indicate a dose-response effect on metabolism. We agree this should be clarified in the design paper, therefore the following sentence has been added on on page 5, line 5:
"A dose of 25 mg rather than 10 mg was chosen, as previous studies have indicted a dose-response effect on metabolic outcomes" citing the following article: **https://doi.org/10.1111/dom.12073.**

4. Is the duration of the intervention (13 weeks) arbitrary or following a specific guideline?

The duration was chosen based on the outcomes of the EMPA-REG study (DOI: 10.1056/NEJMoa1504720). The curves of admission for heart failure and cardiovascular death separated at week 12-13, which was the primary reason for the duration of intervention in the current trial. We thank the reviewer for this comment and have provided the following sentence in the revised version of the manuscript on page 4, line 14:
"The duration of 13 weeks was chosen, as the EMPA-REG study showed a clear effect on the primary outcomes at this timepoint."

5. Is there any termination criteria (e.g. safety grounds)? Tilføjet på side 5

The protocol includes criteria for suspension of the study medication. These criteria have been added to the revised version of the article text (page 5, the section 'Trial Intervention'):

" The study medicine will be suspended if the participant becomes pregnant or withdraws consent. The investigators may suspend the medication for safety reasons."

Reviewer 2

1) The methodology of adipose tissue determination of cytokines for mRNA is not properly presented. How tissue will be extracted and how it will be processed for the gene expression analysis profile. Moreover, why did not authors consider ELISA determination on circulating plasma? Do they think this would not be less invasive and additive in terms of scientific knowledge?

We agree with the reviewer that the methology of adipose tissue function determination should be presented in more details. Therefore, the description of the adipose tissue methology (page 7, line 21) has been expanded. The added text in bold:

"Biopsies will be obtained from the abdominal subcutaneous tissue ***lateral to the umbilicus*** *during a fasting state using the Bergstrom needle technique.* ***Following dissection, the biopsies are washed and snap frozen in liquid nitrogen and stored at -80°C.*** *Total mRNA will be extracted from adipose tissue with commercially available lipid tissue kits to measure* ***the*** *mRNA expression of* ***biomarkers of interest****.* ***Protein expression will be analysed using Western blots****. Quantification of inflammatory cells will be performed according to validated histological procedures.* ***Fibrosis levels will be measured using the Sirius Red stain."***

In addition, we plan to perform analyses of various plasma biomarkers using commercially available kits, some of which are based on ELISA. A list of these biomarkers has been added to the revised version of the manuscript on page 9. However, some of the biomarkers are not specific for adipose tissue, and direct measurement of mRNA expression in the tissue is required. Moreover, we will also perform histological analyses of the adipose tissue in order to assess function and inflammation status.

2) Oral glucose tolerance test. Why did authors not include glucagon determination?

We thank the reviewer for this highly relevant suggestion. Aliquots from the OGTT are kept in a study bio bank at -80^{c}, and glucagon determination will be considered in a in a later sub-study.

3) Did authors consider lactate and troponin-I determination as well? Would it be rationale?

Thank you for these suggestions. Lactate is measured during the hemodynamic examination in the substudy but is not a pre-specified endpoint. We will measure hs-TNT, which we consider equivalent and is available in our laboratory, as part of the measurements of cardiac function, as a biomarker we and others have previously demonstrated that hsTNT is a strong biomarker of myocardial function and outcome in varies population including heart failure (https://doi.org/10.1016/j.amjcard.2012.04.033). This biomarker has accordingly been added to the section "Urine and blood samples, fasting" on page 8.

4) Urine and blood sample determination should be better clarified in the methods. Which are the urine metabolite considered for renal evaluation (beside the instrumental analysis)?

The description of blood and urine sampling on page 8 has been expanded.
The only measurement performed on urine is the albumin/creatinine ratio. A description of this measurement has been added to the text on page 8 and 9.
5) Please, change "pharmaceutical dosage" into "pharmacological dosage".
For placebo: do authors consider similar ingredients for palatability?

a) **The text has been changed from** "pharmaceutical dosage" into "pharmacological dosage".
b) **The active drug, as well as the placebo, are provided as granulate in capsules that are swallowed whole. Since the same type of capsule is used for both substances, there should be no difference in palatability.**

6) Why did authors consider only the 25 mg form? Although the rationale is to estimate maximal empagliflozin on the outcome, would it be more rationale to consider a dose-escalating approach in such a particular study?

We chose the high dose of 25 mg, since the current trial examines the metabolic effects of empagliflozin in secondary endpoints, and previous studies indicate a dose-response effect on metabolism. We agree this should be clarified in the design paper, and the sentence *"A dose of 25 mg rather than 10 mg was chosen, as previous studies have indicated a dose-response effect on metabolic outcomes"* **has been added on page 5, line 5. In addition, this article has been added: https://doi.org/10.1111/dom.12073.**

We did not consider dose-escalation in the current study as we sought to replicate the procedure in the EMPA-REG study, in which the high dose was given immediately.

7) Why did authors not consider a cross-over approach?

A cross-over study has been considered, but we believe that the amount of procedures the participants would have to undergo would be excessive, e.g. patients in the hemodynamics subgroup would undergo heart catherization four times during the study. Thus, we were concerned that the participants would not agree to this protocol, which was the primary reason for not doing the cross-over study.

8) I suggest to implement the time-line design of the study graphically.

Thank you for this suggestion, a time-line of the study visits has been added as a new Figure 1 in the revised version of the manuscript.

9) Pag.6 line 21: why did authors consider adenosine rather than nitro-glycerin? Why did authors consider such dosage i.v.?

A standard clinical protocol is used when performing the Rb82 PET/CT scan. This protocol is used in the daily clinic work the the Department of Clinical Physiology, Nuclear Medicine and PET at Rigshospitalet, and covers the choice of pharmaceutical and doses. Since, adenosine and dipyramidole are the most commonly used vasodilators for this examination in this department we used this available protocol in the SIMPLE trial.

10) Better definition of echocardiography procedure is suggested.

Thank you for this suggestion, and we agree with the reviewer on this item. Therefore, the description of the procedure (page 6, line 26) has been expanded. Added text in bold:
"The following *echocardiographic measurements will be obtained using 3D and 2D imaging: left ventricular ejection fraction (LVEF), left ventricle (LV) end-diastolic and end-systolic diameter, LV mass and left atrial volume. From pulsed-wave Doppler mitral inflow curves, E-wave, A-wave, E-decT and isovolumetric relaxation time will be recorded. Tissue Doppler imaging will be obtained in the apical four-chamber, two-chamber and apical long-axis to evaluate peak systolic (s'), early diastolic (e') and late diastolic (a') velocities during the ejection period. Strain measures of LV (radial, circumferential and longitudinal speckle tracking) will be assessed via 2D echocardiography.* ***The longitudinal tissue velocity of the LV will be assessed by averaging myocardial velocities and displacement of the mitral annular position in the septal, lateral, inferior, posterior and anterior wall of the LV. Diastolic function will be assessed according to EAE/ASE recommendations."***

11) Diabetes disease vintage is roughly considered and explained in table 1. Authors report that patients with diabetes diagnosed at least three months prior to randomization will be included. However, which is the maximal disease duration conceived. It is pivotal to report this point as this will be crucial for outcomes. Do authors believe to find more important effects on ling-lasting diabetes vs early-onset diabetes?

We agree that the duration of diabetes is of great importance, and these data will be included when the study data is published. Diabetes duration is obtained at the screening visit, and the description of this visit has been expanded. The text (page 6) now reads "*At the screening visit (V0), informed consent will be obtained, and patients will be assessed for eligibility based on medical* ***history, including diabetes duration and comorbidities****, physical examinations and blood samples.***" (additions in bold).**
There is no upper limit for diabetes duration in this study, since we aimed that the inclusion and exclusion criteria should be close to the criteria used in the EMPA-REG outcome trial. We

do not know at this time point whether the treatment effect will depend on the diabetes duration, or on the severity of the cardiovascular risk factors.

12) Do authors conceive to measure C-reactive protein as well? What do authors think such potent pharmacological compound, empagliflozin, would add on top of recently proposed anti-inflammatory therapies in high-risk patients (see: Ridker PM JACC 2018; Verma S JACC 2018). Please, comment in light of previous point as well.

We are thankful to the reviewer for bringing these papers to our attention. The paper by Ridker PM et al. JACC 2018 has been added to the reference list.
High sensitivity CRP is measured as part of the blood analysis at V1 and V3 but has not been pre-specified as an outcome. However, we will publish data on the effect on inflammation as part of later sub-studies. We agree that the effects of empagliflozin treatment in conjunction with anti-inflammatory therapies could be of potential intereset, and based on the paper by Ridker, one could speculate that the effects would be additive. We measure several inflammatory biomarkers in the SIMPLE trial, such as IL-6 and TNF-α, and a list of these biomarkers has been added to the text on page 9-10

13) In table 1: "stable dose of anti-diabetic therapy" is elusive: could authors better explain?

The phrase "*stable dose of anti-diabetic therapy***" has been changed "***no change in anti-diabetic therapy within 30 days prior to baseline***" in Table 1, which is less ambiguous.**

Reviewer 3

1) This is a randomized, double blind controlled trial, Please correct the name "DECLAIRE TIMI trials" to DECLARE TIMI trials.

The text has been corrected.

2) What is "MBF" in the second paragraph of the session Statistical Analysis, Sub-study key endpoint?

Thank you for this notion, the abbreviation has been corrected to 'MFR'.

3) Inclusion Criteria:
Hba1c of ≥ 48 mmol/L and ≤ 86 mmol/L at screening for patients on background therapy or Hba1c of ≥ 48 mmol/L and ≤ 75 mmol/L at screening for drug-naïve patients. Why?

Similar to the EMPA-REG study, we wish to include stable patients that are neither severely dysregulated, and therefore need intensification of their treatment regime, nor over-regulated, and therefore at risk of hypoglycemia due to the study intervention. Thus, we aimed that the SIMPLE trial would be comparable with the population in the EMPA-REG study.

4) There are a lot of initials without its description in table 1.

The missing abbreviations have been added, and we thank the reviewer for this comment.

5) Exclusion Criteria:
History of ischemic or hemorrhagic stroke > 2 months prior to informed consent - what about vascular malformation as a cause of hemorrhagic stroke?

Stroke from vascular malformation would be counted a hemorrhagic stroke in the current study, and we agree that this can be considered a weakness of the inclusion criteria. However, due to the rarity of this form of stroke (~1% of all hemorrhagic strokes, https://doi.org/10.1161/01.CIR.103.21.2644), we do not expect that this will affect the results of the study.

6) Your flowchart of study "visits" (table 2) is not consistent with your text ("Trial visits and procedures - Visits"). Please review and rewrite it.

We are thankful to the reviewer for spotting this. The errors in the flowchart have been corrected.

VERSION 2 – REVIEW

REVIEWER	Andrea Baragetti University of Milan, Department of Pharmacological and Biomolecular Sciences. Bassini Hospital, Center for the Study of Atherosclerisis, Cinisello Balsamo, Milan
REVIEW RETURNED	24-Aug-2019

GENERAL COMMENTS	1) The methodology of adipose tissue determination of cytokines for mRNA is not properly presented. How tissue will be extracted and how it will be processed for the gene expression analysis profile. Moreover, why did not authors consider ELISA determination on circulating plasma? Do they think this would not be less invasive and additive in terms of scientific knowledge? R1:ok 2) Oral glucose tolerance test. Why did authors not include glucagon determination? R1:not addressed. 3) Did authors consider lactate and troponin-I determination as well? Would it be rationale? R1:ok. 4) Urine and blood sample determination should be better clarified in the methods. Which are the urine metabolite considered for renal evaluation (beside the instrumental analysis)? R1:ok 5) Please, change “pharmaceutical dosage” into “pharmacological dosage”. R1:OK. For placebo: do authors consider similar ingredients for palatability? R1:OK. 6) Why did authors consider only the 25 mg form? Although the rationale is to estimate maximal empagliflozin on the outcome, would it be more rationale to consider a dose-escalating approach in such a particular study? R1:ok. Is it possible to better add brief explanation in the text about “dose-response effect on metabolic outcomes”? 7) Why did authors not consider a cross-over approach? R1:partially addressed. Although this is a registered study, a better explanation of this point is warranted. 8) I suggest to implement the time-line design of the study graphically. R1:ok. 9) Pag.6 line 21: why did authors consider adenosine rather than nitro-glycerin? Why did authors consider such dosage i.v.? R1:ok. 10) Better definition of echocardiography procedure is suggested. R1:OK.

	11) Diabetes disease vintage is roughly considered and explained in table 1. Authors report that patients with diabetes diagnosed at least three months prior to randomization will be included. However, which is the maximal disease duration conceived. It is pivotal to report this point as this will be crucial for outcomes. Do authors believe to find more important effects on ling-lasting diabetes vs early-onset diabetes? R1: this point is still lacking in study population section. 12) Do authors conceive to measure C-reactive protein as well? What do authors think such potent pharmacological compound, empagliflozin, would add on top of recently proposed anti-inflammatory therapies in high-risk patients (see: Ridker PM JACC 2018; Verma S JACC 2018). Please, comment in light of previous point as well. R1: partially explained. 13) In table 1: "stable dose of anti-diabetic therapy" is elusive: could authors better explain? R1:ok. The purpose of the manuscript is very interesting and results are promising. However, several points warrant better definition and clarification. I therefore suggest a major revision of the manuscript. R1: some minor points are still pending, before manuscript could be supported for acceptance. Thank you for considering my revision.

VERSION 2 – AUTHOR RESPONSE

Response to reviewers

2) Oral glucose tolerance test. Why did authors not include glucagon determination?

R1:not addressed.

We thank the reviewer for this highly relevant suggestion. A description of the effect of SGLT-2 inhibitors on the glucagon-insulin ratio has been added to the manuscript "*SGLT-2 inhibitors have been shown to increase the glucagon-insulin ratio, presumably due to the decrease in glucose stimulus.**"* **(page 4, line 8), and a reference has been added (Ferranini, Cell Metabolism 2017) Aliquots from the OGTT are kept in a study bio bank at -80^{c}, and glucagon determination will be measured in a in a later sub-study. The sentence "**Frozen aliquots for measurement of glucagon will be kept for a later sub-study." **has been added to the OGTT description in the Methods section of the manuscript (page 8, line 17)**

6) Why did authors consider only the 25 mg form? Although the rationale is to estimate maximal empagliflozin on the outcome, would it be more rationale to consider a dose-escalating approach in such a particular study?

R1:ok. Is it possible to better add brief explanation in the text about "dose-response effect on metabolic outcomes"?

The description has been expanded and the dose-dependent effects are stated (page 5, line 5, additions in bold):
"A dose of 25 mg rather than 10 mg was chosen, as previous studies have indicated a dose-response effect on metabolic ***outcomes such as urinary glucose excretion, fasting plasma glucose and mean plasma glucose, with higher drug doses resulting in lower glucose levels and increased glucose excretion.****"*

Dose-escalation was not considered in the current study as we sought to replicate the procedure in the EMPA-REG outcome trial, in which the high dose of 25 mg was given immediately. This explanation has been added to the study manuscript in the Trial Intervention section: "*In accordance with the EMPA-REG study, no dose-escalation will be performed."* **(page 5, line 16)**

7) Why did authors not consider a cross-over approach?

R1:partially addressed. Although this is a registered study, a better explanation of this point is warranted.

As stated in the previous review, we did consider the cross-sectional design, since the SD on the efficacy parameters is reduced, and since the required sample size is smaller than in the parallel randomized design. However, we did have a large type 2 diabetes outpatient clinic and was confident that we would be able to enroll the 92 patients required for the parallel randomized trial. The sentence "A cross-over design was considered but not implemented, due to the expected high availability of eligible participants." **has been added to the manuscript (page 5, line 14).**
A cross-over approach would necessitate that patients participate for at least 7-8 months, while undergoing four invasive examinations in the hemodynamic substudy as well as four adipose tissue biopsies, four PET/CT scans etc.
In addition, the design of a cross-over study would have required a wash out period between the study arms and we were concerned of the lengths of this washout, in order to be sure not to introduce a carry-over affect. The length of the study would thus be expanded with a potential larger risk of drop outs. Therefore, we decided to do the classic parallel design RCT. We hope this is a satisfactory explanation for choosing the present design of the trial.

11) Diabetes disease vintage is roughly considered and explained in table 1. Authors report that patients with diabetes diagnosed at least three months prior to randomization will be included. However, which is the maximal disease duration conceived. It is pivotal to report this point as this will be crucial for outcomes. Do authors believe to find more important effects on ling-lasting diabetes vs early-onset diabetes?

R1: this point is still lacking in study population section.

The first sentence of the study population section has been modified to elucidate this point (page 5, line 7, additions in bold):
"The study will include 92 patients ***who have had the T2D diagnosis for at least 3 months, and with no upper limit to the duration of diabetes****, who have either additional CV risk factors or pre-existing CVD."*

12) Do authors conceive to measure C-reactive protein as well? What do authors think such potent pharmacological compound, empagliflozin, would add on top of recently proposed anti-inflammatory therapies in high-risk patients (see: Ridker PM JACC 2018; Verma S JACC 2018). Please, comment in light of previous point as well.

R1: partially explained.

We thank the reviewer for suggesting the excellent paper by Verma S (JACC 2018). The paper has been added as a reference to the segment on ketone bodies, which has been expanded: "*Moreover they induce a small increase in plasma ketone bodies, which some have proposed to be a preferred fuel source for the myocardium.* ***However, others have shown that SGLT-2 inhibitors increase ATP production in mouse model hearts, without increasing ketone oxidation****."* **(page 4, line 9, additions in bold)**