PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Investigating the roles of hyperglycemia, hyperinsulinemia, and elevated free fatty acids in cardiac function in patients with type 2 diabetes via treatment with insulin compared with empagliflozin: protocol for the HyperCarD2 randomised, crossover trial
AUTHORS	Thirumathyam, Roopameera; Richter, Erik; Goetze, Jens; Fenger, Mogens; Van Hall, Gerrit; Dixen, Ulrik; Holst, Jens; Madsbad, Sten; Vejlstrup, Niels; Madsen, Per; Jørgensen, Nils Bruun

VERSION 1 – REVIEW

REVIEWER	Ele Ferrannini University of Pisa School of Medicine, Department of Internal Medicine
REVIEW RETURNED	24-Sep-2021

GENERAL COMMENTS	The study proposed in this MS addresses an important question, will use state-of-the-art techniques (including repeat biopsies), and will be performed by expert, highly capable investigators. Its results will add significantly to our current understanding of human cardiac metabolism. Regarding the protocol, I have the following considerations: 1) Why use a 5-hour OGTT instead of the more physiological mixed meal tolerance test? 2) The cross-over design is statistically efficient but with 20 participants it does expose the protocol to carry-over bias and patient drop-out. 3) Though CMR is quite reproducible, I would worry that 5 weeks of active treatment may not be sufficient to change the primary outcome detectably in patients with essentially normal contractile function (cf. Oldgren J et al. Diabetes Obes Metab. 2021;23(7):1505-1517.

REVIEWER	Marcus Säemann Sigmund Freud Private University Vienna
REVIEW RETURNED	02-Jan-2022

GENERAL COMMENTS	In their manuscript Thirumathyam et al. present their study protocol to assess cardiac functionality in cardiovascular high-risk patients with type 2 diabetes mellitus, the primary endpoint will be the difference of left ventricular peak filling rate determined by MRT (w/o stress conditions). In short, by means of a randomized crossover study, the effects of SGLT-2 inhibition versus exogenous insulin treatment will be studied based on the premise that these antidiabetic therapies substantially differ regarding their effects on fatty acid and overall energy metabolism in heart and skeletal muscle tissue. This study is of high importance as it may help to

	better understand the underlying metabolic and molecular mechanisms underlying the well-known cardioprotective effects of SGLT-2i. Major points: A cross-over trial principally stands on the assumptions that treated subjects in one arm return to their initial biological state after the treatment is over including the wash-out period. Is this necessary assumption potentially violated with the study protocol or is taken for granted with the given treatment and wash-out periods that all parameters are reversible and revert to their initial state? Any possibilities that this could be the case should be at least discussed. Since the possibility that ketone bodies could be energetically utilized in SGLT-2i-treated subjects is studied: I could not find beta-hydroxybutyrate measurements in the protocol; will they be performed and at which time points? Will there be an assessment of the macronutrient intake of the patients? Can it be assured that this will not change during the study? For example, a significant reduction of carbohydrate intake during empagliflozin treatment could booster ketone body production possibly increasing the observed difference of measured parameters. In the same direction goes the question whether physical activity of the participants will be assessed during the study as it also may influence especially diastolic function. Is there an ideal HbA1c range for participants apart from the exclusion of > 9%? Was CGM considered? What about blood pressure control and control of fluid status? SGLT-2i reduce blood pressure and could thereby influence the results as well as via their diuretic effects especially cardiac functional parameters Minor points: As often, it would be helpful for the reader – especially regarding cross-over studies – to have an illustration to understand all measures at one glance, hence could figure one be enhanced with the examinations planned? This prevents unnecessary regarding throughout the manuscript to understand a particular detail. Is there a control, whether reduction of free fatty acids alone (acipimox without empagliflozin) influences cardiac measures?

VERSION 1 – AUTHOR RESPONSE

Reviewer #1
Why use a 5-hour OGTT instead of the more physiological mixed meal tolerance test?

Answer: We agree that a meal test is more physiological than an OGTT because the intestinal hormones, insulin and glucagon are stimulated by daily-life macronutrients and not only by glucose as during an OGTT. The problems with the mixed meals is that the macronutrient composition differs which makes it difficult to compare results between studies. This is less of a problem when comparing results between studies using an OGTT, where we still have the secretion of the hormones including the incretins, insulin and glucagon. Additionally, we chose the OGTT over a mixed meal because we use an oral glucose tracer technique. It is easier to distribute the tracer equally in an oral glucose load than in a solid mixed meal.

Change in MS: None.

The cross-over design is statistically efficient but with 20 participants it does expose the protocol to carry-over bias and patient drop-out.

Answer: We chose the cross-over design for statistical efficiency. As pointed out, this design carries a risk of carry-over effects, but in a previous study performed in collaboration with our department, where T2D patients were subject to 12 weeks of liraglutide treatment, no carry-over effect was detected after 14 days of washout [1]. Here the intervention is shorter, and washout is longer, and the effect on body weight and glycaemic control between the treatments is expected to be minimal, therefore the risk of carry-over effects is deemed very low. However, with two baseline visits, one before each treatment, we are able to account for any difference in baseline characteristics resulting from the treatment order. This greatly reduces the risk of any carry-over effects and justifies our design. The design with cardiovascular and metabolic tests repeated 4 times is vulnerable to drop-out, as mentioned, but the risk of drop-out often depends on the investigators' care and contact with the participants, and we hope that we will succeed in keeping the participants in the study by frequent phone contacts.

Change in MS: We have updated the power analysis with a scenario of a dropout rate of 20%, which still leaves us with a decent chance of detecting a difference between the treatments. We have also added this point to the box regarding strengths and limitations.

"In case of a 20% dropout rate, power would still be acceptable (83%, p=0.01)."

"STRENGTHS AND LIMITATIONS

- A cross-over over design is more vulnerable to dropout, but provides greater statistical power"

Though CMR is quite reproducible, I would worry that 5 weeks of active treatment may not be sufficient to change the primary outcome detectably in patients with essentially normal contractile function (cf. Oldgren J et al. Diabetes Obes Metab. 2021;23(7):1505-1517.

Answer: An interesting question. How long should patients with T2D be treated for to obtain a clinical benefit of SGLT2 inhibitors (SGLT2i)? A very short time it seems from the Empa Reg study [2]. We know that 4-5 weeks of treatment result in distinct changes in metabolism [3]. We aim to investigate whether these metabolic changes are associated with changes in cardiac function in patients with type 2 diabetes. And while patients will have essentially normal contractile function in the present study, our primary endpoint is left ventricular peak filling rate, a reproducible measure of cardiac diastolic function. Diastolic dysfunction is an early and very frequent cardiac manifestation of T2D, it is coupled to cardiac metabolism and is a predictor of cardiovascular mortality [4–6].

Changes in metabolic status may have immediate effects on cardiac function both in patients with and without heart failure. For instance, an acute physiological increase in ketone body concentrations improves cardiac function within hours in patients with heart failure [7], and depleting plasma of free fatty acids impairs cardiac function within hours in patients with T2D and normal contractile function [8]. On the other hand longer-lasting [months] SGLT2i treatment may cause structural changes to the myocardium, that are not necessarily associated with changes in metabolism [9,10]. Improvements in diastolic function found with SGLT2i treatment after longer treatment duration [11] are therefore difficult to ascribe to direct metabolic effects of the treatment alone. At the same time, extending the study period would make the design much more susceptible to dropout and carry-over bias.

We have chosen 4-5 weeks of treatment to make sure metabolic changes are present while at the same time reducing structural changes to the heart. But to really force the metabolic changes on the heart, we not only compare effects of treatment on cardiac function to washout, but also to insulin

treatment, which from a metabolic perspective lowers glucose in a manner opposite to empagliflozin (Low FFA and ketone bodies, high insulin), AND we investigate cardiac function during the two treatments and during treatment + acute depletion of FFAs/ketone bodies.

Thus, if metabolic changes with SGLT2i treatment do indeed play a major role in cardiac function in patients with type 2 diabetes, we feel confident that we would be able to show it in the present study design.

Change in MS: We have changed the background section slightly to better reflect these arguments and updated the references [Nielsen R et al Circulation 2019]

“An early and interesting hypothesis proposed that changes in cardiac metabolism may be responsible for the cardioprotective effect of SGLT2i. The lowered glucose and insulin concentrations, persistent hyperketonaemia and elevated free fatty acids, caused by SGLT2i treatment, leads to reduced glucose uptake, increased ketone body uptake and oxidation and unchanged uptake of free fatty acids in the heart while overall lipid oxidation is increased [37,38]. This altered energy metabolism may rapidly improve myocardial function, especially during myocardial stress [39–42].”

Reviewer #2

A cross-over trial principally stands on the assumptions that treated subjects in one arm return to their initial biological state after the treatment is over including the wash-out period.
Is this necessary assumption potentially violated with the study protocol or is taken for granted with the given treatment and wash-out periods that all parameters are reversible and revert to their initial state? Any possibilities that this could be the case should be at least discussed.

Answer: Again, a relevant question! This point has also been addressed by reviewer 1, and we kindly refer to our answer to his question.

Since the possibility that ketone bodies could be energetically utilized in SGLT-2i-treated subjects is studied: I could not find beta-hydroxybutyrate measurements in the protocol; will they be performed and at which time points?

Answer: A very relevant point! We have not explicitly mentioned beta-hydroxybutyrate measurements in the original manuscript, but we have written “ketone bodies” in Box 5 describing the biochemical measures planned.

Change in MS: We have now added beta-hydroxybutyrate to text box 5.

Will there be an assessment of the macronutrient intake of the patients? Can it be assured that this will not change during the study? For example, a significant reduction of carbohydrate intake during empagliflozin treatment could booster ketone body production possibly increasing the observed difference of measured parameters. In the same direction goes the question whether physical activity of the participants will be assessed during the study as it also may influence especially diastolic function.

Answer: We have not designed the study to account for nutrient intake. Participants will be given general dietary advice in relation to phone contacts. We will account for blood glucose in the washout and treatment periods, fasting time preceding metabolic and cardiac MRI study days is also

harmonized, but nutrient composition is not controlled for. In terms of boosting ketone body production by changing dietary pattern, that would be helpful to the study, since we would prefer greater metabolic differences between the two treatments. Regarding exercise, it is true that being more physically active during one treatment could bias towards better diastolic function, however, although we do not have any accelerometer data planned, participants are fitted with a 48-hrs Holter monitor. Any increased activity would reflect in periodically increased heart rate. Additionally, we test VO2 max. Any substantial and systematic increase in physical activity should reflect in an increased VO2 max. We are therefore confident, that any such bias will be detected.

Change in MS: None

Is there an ideal HbA1c range for participants apart from the exclusion of > 9%? Was CGM considered? What about blood pressure control and control of fluid status? SGLT-2i reduce blood pressure and could thereby influence the results as well as via their diuretic effects especially cardiac functional parameters

Answer:
Regarding ideal HbA1c range: There are no further limitations than those already described.

Regarding CGM: Has been considered, but not included in the protocol, mostly for logistical reasons. CGM sensors should be changed every 10-14 days. In a study like this, where there are many clinical contacts, it is important to reduce visits to study site, because this increases the risk of dropout. In that perspective, we chose finger stick glucose measurements because it is a well proven technology, that has a low failure rate.

Regarding blood pressure and fluid state: We do not control for either in the present study. However, we account for any changes in BP with a 24-hrs ambulatory BP measurement on top of study day blood pressures. With respect to fluid state, an exploratory endpoint is central blood volume, which should change with fluid status. So, while not controlling for fluid state in the study, we do account for changes. However, if metabolic changes do play a role, we would expect a differential response to acipimox during the two treatments independently of these factors.

Change in MS: None

As often, it would be helpful for the reader – especially regarding cross-over studies – to have an illustration to understand all measures at one glance, hence could figure one be enhanced with the examinations planned? This prevents unnecessary regarding throughout the manuscript to understand a particular detail.

Change in MS: We have enlarged the figure and added the study points in a more reader friendly manner. In the figure text, we have referred to text box 4, where test performed at each visit are summarized

“Figure 1. Study outline. Included patients undergo a 7-week program of washout of pre-existing antiglycemic treatment (except metformin) and run-in of empagliflozin. Hereafter they are randomized to treatment for 5±1 weeks, followed by 3±1 weeks wash-out and cross-over to 5±1 weeks treatment with the remaining study drug. Tests performed at each visit are summarised in Box 4 and 5.”

Is there a control, whether reduction of free fatty acids alone (acipimox without empagliflozin) influences cardiac measures?

Answer: The design with baseline (washout) visits prior to each treatment period, where patients undergo a full metabolic and cardiac examination without any treatment, provides the basis for control.