KETO-CHF

Project protocol

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Modulation of circulating levels of the ketone body 3-hydroxybutyrate in patients with chronic heart failure: Cardiovascular and metabolic effects

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1 Background

Heart Failure (HF) is a major public health issue because the disease affects 1-2% of the Western population[1] and the lifetime risk of HF is 20% [1]. HF is responsible for 1-2% of all healthcare expenditures and 5 % of all hospital admissions [2]. The cornerstone in the medical treatment of chronic HF is a combination of ACE-inhibitors/ATII-receptor antagonists, beta-blockers and mineralocorticoid receptor antagonists [3]. Despite major improvements in the management and care of patients with HF, the 1-year mortality in patients with HF is 13 % [4] and >50% of HF-patients is admitted during a 2.5 year period [5]. Furthermore patients with HF have markedly decreased physical capacity and quality of life. Thus, there is a need for new treatment modalities in this group of patients.

Ketone bodies are produced in the liver and are of vital importance in the human body for energy generation in the heart and brain during fasting, exercise and severe illness [9, 10]. However, ketosis can be safely obtained using dietary supplements [10-12] and can increase exercise capacity in athletes [6]. The most important ketone bodies are 3-OHB and acetoacetate. Recently, it was demonstrated that patients with severe HF have increased myocardial utilization of the ketone body 3-hydroxybutyrate (3-OHB) [7]. It has been hypothesized that ketone bodies may act as "superfuel" for the failing heart [8]. In support of this, the glucose-lowering SGLT-2 inhibitor empagliflozin reduces the risk of hospitalizations and cardiovascular death in diabetic patients with HF [9] and also increases circulating levels of 3-OHB [10].

We have shown, using positron emission tomography, that ketone body infusion reduces myocardial glucose uptake and increases myocardial blood flow in healthy subjects [11]. Data from another study conducted by our group show a 40% increase in cardiac output during infusion of 3-OHB [12]. The mechanisms behind these marked hemodynamic effects are presently unknown, but could involve prostaglandin-release. 3-OHB is the endogenous ligand for the G protein-coupled receptor hydroxy-carboxylic acid 2 (HCA2) receptor. This receptor has downstream effects on cAMP and systemic effects via release of prostaglandins [13].
Presently there are no data on the clinical cardiovascular and metabolic effects of long - or short term oral ketone-supplementation in patients with chronic HF.

2 Hypotheses

Exercise capacity and cardiac function can be improved in patients with chronic HF by increasing circulation ketone bodies. This can be achieved through a dietary supplement of 3-OHB.

3 Objectives

1: To determine uptake kinetics of oral 3-OHB supplements in patients with chronic HF.
2. Furthermore to investigate acute pharmacodynamic effects (i.e. hemodynamic effects) of oral 3-OHB intake by two different ketone-supplements.
3: To investigate the effect of 4 weeks modulation of circulating ketone body levels on cardiac function and exercise capacity in chronic HF patients.

4 Design and endpoints

The study consists of one pilot study (KETO-KINETICS) and one main study (KETO-CHF) with a hemodynamic substudy and a metabolic substudy. . The purpose of the pilot study is 1) to establish uptake kinetics and 2) acute hemodynamics of two oral ketone dietary supplements, and 3) differentiate acute and chronic effects of oral ketone supplementation. There will be separate participant informations and consent forms for the substudies. Thus participants can opt only to participate in one substudy if they prefer. The study days are outlined in table 1-3.

KETO-KINETICS: KETO-CHF pilot study:
Design: 10 patients are studied after an overnight fast, in random order on 3 visits, during a) oral intake of 36 g Perfect Keto Oral 3-OHB supplement, b) oral intake of 25 g HVMN Ketone Ester, and c) during a placebo isocaloric drink (similar energy content as the 36 g 3-OHB dose, containing 1:1:2 mixtures of dextrose, fructose, and maltodextrin) [6].
Endpoints: Cardiac output, venous 3-OHB levels, LVEF, LV global strain.
Methods: Swan-Ganz Monitoring, Echocardiography, ECG and blood pressure will be recorded hourly. 3-OHB levels are measured by venous blood samples every 60 minutes. Sampling will be performed hourly from 1 hour before till 5 hours after oral intake.

KETO-CHF: Randomized, double blind crossover study with 20 stable chronic HF patients.
Design: Patients are studied after 4 weeks of oral 3-OHB three times daily(36 g "Perfect Keto Base" or 25g HVMN Ketone Ester depending on the outcome of KETO-KINETICS three times) and 4 weeks isocaloric placebo-drink. Both "Perfect Keto Base" (Perfect Keto, Glendale Heights, IL, USA) and HVMN Ketone Ester (HVMN, San Francisco, CA, USA) are a commercially available ketone diet supplements.
Endpoints: 1) Cardiac output (primary), mixed venous saturation (SVO2), pulmonary wedge pressure 2) Rest/Peak Exercise Echocardiography (LVEF, Global Strain), 3) Cardiopulmonary exercise test, 4) Whole-body substrate metabolism, insulin signalling and lipolysis.
Methods: Swan-Ganz monitoring, echocardiography, exercise capacity, whole-body glucose, FFA and protein metabolism will be examined using isotope tracer techniques. Furthermore, muscle biopsies will be drawn for studies of insulin signalling and lipolysis (chapter 5).

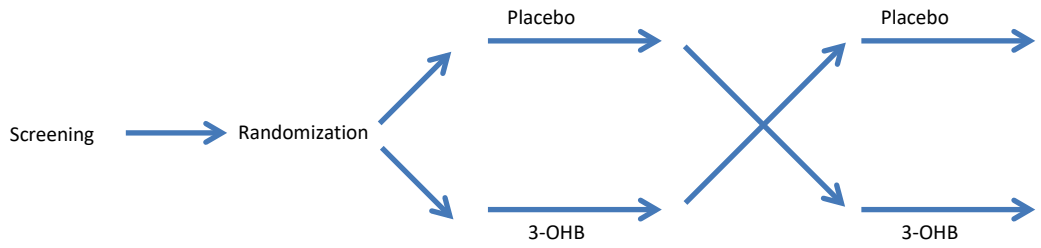

Figure 1: Patient Inclusion flowchart

Table 1: Study Visits Overview

Visit	**V0** **Screening**	**Teleph.** **Contact**	**V1** **Randomization**	**V2** **Hemodynamic examination 1**	**V3** **Metabolic Examinations 1**	**V4** **Hemodynamic examinations 2**	**V5** **Metabolic Examinations 2**
Time (**D**ays, **W**eeks, **M**onths)	**- 4W** to **0**	**-3½ W-0W**	**0**	**3-5W**	**3-5W**	**10-12W**	**10-12W**
General							

Informed consent	X						
In-and exclusion criteria	X		X				
Relevant medical/ surgical history			X				
Relevant concomitant medication (1)			X				
Randomize patient			X				
Study medication							
Delivery	(Trial period)		X				
Crossover					X		
Clinical assessment							
NYHA class			X	X	X	X	X
Height			X				
Weight			X	X	X	X	X
Echocardiography				X		X	
Swan-Ganz Monit.				X		X	
CPX-test				X		X	
Whole-Body Metabolism					X		X
Blood Samples				X	X	X	X
Muscle Biopsies					X		X
ECG			X	X		X	
Blood pressure, heart rate	X		X	X	X	X	X
Safety							
Reporting of: -Endpoints -SUSARs -selected AEs		X	X	X	X	X	X

Table 2: Hemodynamic study day overview

Time	**07.30**	**08.30**	**09.15**	**09.30**	**10.30**	**10.50**	**11.10**	**11.30**	**12.30**
Arrival	x								
Swan Ganz		x							
Exercisetest					x				
Blood Samples			x		x	x	x	x	
Echocardiography			x		x	x	x	x	
Invasive measurements			x		x	x	x	x	

3-OHB/placebo ingestion	x
End of study day	x

Table 3: Metabolic study day overview

Time	**07.30**	**08.30**	**09.30**	**10.30**	**11.30**	**12.30**	**13.30**	**14.30**
Arrival	x							
Basal period								
3-OHB sipping								
3-OHB sipping + Clamp								
[3-^{3}H]Glucose-Infusion								
[9,10-^{3}H]Palmitate-infusion								
Muscle Biopsy			x				x	
Blood samples	x		x	x	x	x	x	

5 Methods

5.1.1 Swan-Ganz Monitoring:

This is a standard procedure at the clinical research site and will be performed in a dedicated cardiac catheter lab with experienced physicians. Standard triple-lumen Swan-Ganz thermister and balloon-tipped catheters will be used. Catheters will be introduced using the internal jugular vein, using ultrasound guidance, and advanced using pressure waveform and fluoroscopy into the pulmonary artery. Cardiac output will be measured using the thermodilution method. Measurements will be made at rest, during peak exercise and post-exercise.

5.1.2 Rest/Peak Exercise Echocardiography:

We intend to use a GE VIVID 7 or 9E system (GE Medical System, Horten, Norway) with a 2.5-MHz transducer for transthoracic echocardiography. For image analysis EchoPAC (GE-Vingmed Ultrasound, Horten, Norway) will be used. All measurements will be the averaged over 3 consecutive heart cycles in end-expiration. 2D echocardiography will be performed according to national guidelines [14]. In case of poor image quality, a contrast agent (SonoVue/Optison) will be used to opacify the left ventricle (LV) [15, 16]. Regarding LV measurements, the following parameters will be recorded: 2D [16], regional wall motion score (16 segment model), LVEDV and LVESV, left atrial volume, tissue Doppler study of the mitral annulus and LV strain and strain-rate[17].

5.1.3 Whole-body metabolism:

Glucose metabolism is determined by isotope dilution technique with constant infusion of [3-3H] glucose (12 µCi / min) from two hours before and 60 minutes after intake of ketones or isocaloric placebo.

FFA Turnover is determined by isotope dilution technique with 2 times 1-hour constant infusion of [9.10-3H] palmitate (0.3 µCi / min, totalling 36 µCi) before and 60 minutes after oral intake. Blood samples to determine FFA concentration and specific activity will be taken at baseline and after 30, 40, 50 and 60 minute infusion. Plasma FFA (palmitate) concentration and specific activity are determined by HPLC[18].

5.1.4 Muscle biopsies

Samples are taken from the vastus lateralis by sterile technique. Proteins involved in insulin-signalling and lipolysis will be determined by Western Blot technique.

5.1.5 Cardiopulmonary Exercise Test:

Cardiopulmonary exercise capacity will be performed using a staged bicycle exercise test with measurements of oxygen consumption and carbon dioxide excretion. After resting measurements are obtained patients will start exercising at 0 watts and using 3 minute intervals increase to 20 watts and then with 10 watt increments every 3 minutes until peak exercise (Borg > 18). At each level of exercise hemodynamic and echocardiographic measurements will be recorded. A preliminary exercise test will be performed prior to the baseline study to make the patient familiar with the procedure.

5.1.6 Blood samples:

Venous blood samples for measurements of plasma insulin, free fatty acids, glucose, ketone bodies, lactate, haemoglobin, P-Na^+, P-K^+. Will be measured three times during each visit P-LDL, P-HDL and P-Cholesterol (total), prostaglandin E2, I2, F2α, thromboxane A, erythropoietin, thrombopoietin and granulocyte stimulating factor will be measured twice during the pilot study and the hemodynamic study days.

6 Power calculation and statistics

Power calculations

In the pilot study we plan to include 8 patients. However, if necessary because of drop-outs or missing values up to 10 patients will be included.

Coefficiency of variation (CV) regarding Cardiac output by pulmonary artery catherization is determined to be 4 % [19]. By enrolling 8 patients in the KETO-KINETIC pilot study, we will be able to detect a relative difference of 6% with a power of 90% and a two-sided significance level of 5%

In the KETO-CHF study by enrolling 20 patients in the hemodynamic examinations, and allowing up to 20 % missing values, we will be able to determine a relative difference of 4 %, and thus detect changes related to clinical outcome [20], with a power of 90% and a two-sided significance level of 5%.

In the metabolic studies, previous studies have shown an SD of the difference of 8% and an effect size of 10%. With power and significance level as described above a sample size of 8 patients are needed. To allow 20% missing values 10 patients will be included in the metabolic studies.

Reporting of endpoints:

For these studies normally distributed variables will be presented as mean±SD. Non-parametric statistics and appropriate log-transformation will be performed if assumption of normality is not met. A two-tailed p value of 0.05 or less will be considered statistically significant.

Differences will be compared by appropriate paired statistical tests. Regression analysis will be performed and correlation coefficients will be presented when appropriate.

7 Patient recruitment

Patients with stable HF will be recruited from the outpatient HF clinic at Aarhus University Hospital.

Inclusion criteria: Chronic HF: NYHA class II-III, left ventricular ejection fraction (LVEF) <40%

Exclusion Criteria: Diabetes or HbA1c >48 mmol/mol, significant cardiac valve disease, severe stable angina pectoris, severe comorbidity as judged by the investigator, inability to give informed consent

Recruitment and informed consent

Study participants will be recruited from the participating centres. Participants will receive oral and written information regarding the study together with the brochure: " Volunteer's rights in a health science research project" ("Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt") and an agreement form requesting accept to be contacted by the investigators for further information. Interested subjects will have to sign the enclosed agreement form and return it before being contacted. Subjects agreeing to receive further information will be contacted by phone. The subjects are informed about the project's background, objectives, methods, risks, side effects, disadvantages, benefits and potential usefulness, and that there may be unforeseen risks and impacts of participation in the project. The subjects are informed that participation is voluntary and that he / she at any time without reason can withdraw their consent and drop out of the project without any consequences for their future treatment. The information is repeated orally upon arrival for the screening visit. The subjects will have the opportunity to ask questions and to bring a companion at this visit. The information conversation will take place in privacy in an investigation room at Department of Cardiology, Aarhus University Hospital and carried out by any of the investigators. Before signing the consent form, the participant will have the opportunity to re-consider for at least 24 hours. Copies of participant information and consent form will be given to the subjects. No study-related examinations will be conducted until after the informed consent form has been signed. The investigator will ensure that the participant is adequately informed both orally and written about the study, background, design, risks, side effects, disadvantages, benefits and usefulness. The investigators are responsible for ensuring obtained written informed consent from all subjects before enrolment in the study.

Information on individual medical data will be given by a medical doctor affiliated with the study. However, the individual subject's desire not to know his or her own data will be respected.

By participating in the study, subjects agree that relevant information from medical records that potentially can influence on the study may be passed to the responsible investigators. This may be information relating to medicine intake and previous and current diseases and treatments.

8 Data collection and processing

Data collection and processing will be carried out in accordance with current legislation on processing of personal data, and the general data protection regulation.

Source data will be recorded in the patient's electronic patient record or on specific worksheets. Invasive hemodynamics and echocardiograpic data will be stored at the Department of Cardiology Aarhus University Hospital. A centralized electronic Case Report Form (CRF) will be constructed for data capture. Data will be stored in coded form in 15 years according to recommendations from the Data Monitoring Board. After which it will be transmitted to the Danish Data Archives.

Biobanks

A research biobank will be established at Department of Endocrinology and Metabolism, Aarhus University Hospital. This research biobank will store blood samples in coded form for later analysis (>7 days). Blood samples will be stored at -80°C. After completion of the project, excess biological material (blood plasma and muscle biopsies) will be transferred to a biobank for future research. This biobank will be registered separately with the Central Denmark Region.

9 Patient discomfort and risks

The enrolled volunteers should expect to spend approx. 1 hour at the screening visit and 6-8 hours at each study visits. Participants will have their travel expenses from participating in the project reimbursed. In case of injury, patients are covered under The Patient Compensation Association.

Blood samples (all studies)

The studies will require venous puncture which may inflict a short pain and a risk of a small hematoma. There is a minimal risk for infection by the point of insertion. A total of 340 mL of blood will be drawn for the KETO-KINETICS study distributed over the three study days. In the KETO-CHF 110 mL of blood will be drawn at each visit. This should cause no side effects.

Radiation

The risk for adverse health effects from cancer is proportional to the amount of radiation dose absorbed and strongly dependent on age. When reviewing the numbers below, the natural incidence of fatal cancer in Denmark of about 25% and the 5-year mortality of 43% in Danish heart failure patients [2] should be taken into account. Radiation doses for the KETO-KINETICS and Keto-CHF study is 6 mSv and 4 mSv each respectively, corresponding to IRCP category IIb. Thus, participation in one study increases cancer risk from 25% to 25.02% in the KETO-CHF study and 25.03% in the KETO-KINETICS study.

Right heart catherization

This method has been implemented at our centre for decades both in daily practice and for research use. The applied local anaesthetic, may cause a short stinging pain (few seconds) and in some cases cause temporally hoarseness (1-2 hours). Catheter placement through the internal jugular vein will be performed under sterile conditions. The catheter will be advanced to the pulmonary artery for measurements of pressure and cardiac output. Accidental puncture of the carotid artery will be avoided by transcutaneous ultrasound guidance. A single dose of antibiotics will be given when appropriate (pacemakers, artificial valves, etc). At the puncture site of the neck, a small hematoma can appear which does not require any further treatment. The procedure will be performed by experienced physicians, and according to the guidelines from the Danish Society of Cardiology the complication rate is described as particularly low. Brief periods of atrial fibrillation or self-limiting ventricular arrhythmias are the most common. There are reports of right atrial or right ventricle perforation using stiff catheters, but no such episodes were reported using balloon tipped catheters. Such balloon tipped catheters will be applied in the present project. In a study of 7218 patients undergoing right heart catherization, a fatal episode related to the procedure was described in one patient due to rupture of a pulmonary artery [21]. This patient had severe pulmonary hypertension but no HFrEF. Thus, a patient category with a completely different disease located in the pulmonary vessels. No such patients will be enrolled in the present study. Finally, it should be mentioned that the method is applied in an ongoing trial (TEMPO) at Dept. of Cardiology Aarhus University, and in a recent published study in which healthy Danish volunteers were investigated, and no adverse events were reported [22].

Echocardiography

Echocardiography uses standard ultrasound techniques to image the heart. It is performed with the patient in the left lateral position and is without risk or discomfort for the patient.

Muscle biopsies
During the metabolic examination days two muscle biopsies will be drawn. A local anaesthetic will be used to minimize patient discomfort. After the biopsy, a hematoma will develop as well as soreness of the muscle. In rare cases a local infection may occur. No patients on oral anticoagulant treatment will be subjected to this procedure.

Adverse Effects of study medication
Ketone supplements may cause mild gastrointestinal discomfort and mild headaches in the first days entering a regimen. Patients are therefore subjected to a four day run-in period (2 days of KETO-base supplement and 2 days of placebo) before randomization. Only those able to tolerate side-effects will be included in the study. In case of unacceptable side-effects during the study period, doses will be reduced step-wise until reaching no – or accepted level of side-effects. Blood samples for determination of K^+, Na^+, Ca^{2+} and Mg^{2+} will be drawn after two- four days of treatment and 1-2 weeks later during the study period in the first 6 patients in the KETO-CHF study. If deemed necessary by the investigators this procedure will be carried out in the remaining 14 patients as well.

Potential benefits for the participants
Participants will potentially benefit from the 3-OHB supplement applied in this study. Participants with HFrEF will be evaluated for their present cardiac status. The right sided heart catherization is the gold standard to obtain measures of cardiac output and importantly cardiac filling pressures (preload) conditions. During daily practice, HFrEF patients often have their heart failure medication adjusted after right sided heart catherization as non-invasive assessment of preload conditions are often very difficult or impossible to obtain. Thus, each HFrEF participant will benefit by having their heart failure and diuretic treatment re-evaluated and eventually adjusted or intensified. Furthermore, the study could contribute with pivotal information on a potential future treatment of patients suffering from HFrEF, and determine whether a clinical trial of long-term hyperketonaemia should be initiated.

10 Ethical considerations
The project will be carried out in accordance with the principles of the Helsinki Declaration II. The protocol, including the written participant information and consent forms, must be finally approved by the Research Ethics Committee of the Central Denmark Region and the Danish Data Protection Agency. Data obtained from the subjects are protected under the Act concerning the processing of personal data and health law. The study will be conducted according to the Good Clinical Practice guidelines and will be reported to clinicaltrials.gov before initiation. The study does not need approval from the Danish Medicines Agency since the patients do not receive a drug but a dietary intervention (LMST journal number: 2018110888).

Based on the previously mentioned literature, the overall risk of side effects when participating in these studies is estimated to be relatively low. The results of these studies will contribute with new and essential knowledge of HFrEF and potentially new treatment approaches. Therefore, the investigators are convinced that the possible risks and side effects are outweighed by the expected benefits from the conduct of this study.

11 Collaborators and feasibility
Dr. Kristian Hylleberg Christensen (KHC) is already trained in echocardiography and management of acute and chronic HF. KHC will be trained in imaging techniques needed for the study by the

research group. KHC will be the principal investigator in this study. Dr *Roni Nielsen* (RN) defended his PhD thesis on myocardial metabolism in heart failure in 2013. He has continuously been working with heart failure, cardiac PET imaging and metabolism and has experience with all applied methods. Associate professor *Henrik Wiggers* (HW) and Professor *Hans Erik Bøtker* (HEB), Dept. of Cardiology are specialists in heart failure; they both have bridged the gap between cardiology and metabolism and have considerable experience in the field of myocardial PET. Professor *Niels Møller* (NM), Dept. of Endocrinology and Medicine, is an expert in human *in vivo* metabolism and clinical nutrition and has considerable administrative experience. He has directed similar projects and has a formal education in management and administration. Consultant physician *Lars Gormsen* (LG), Dept. of Nuclear Medicine, has major experience in scientific and clinical interpretation of myocardial PET imaging.

This project is initiated by the multidisciplinary research group, and the proposal could not be conducted without the added synergistic contributions from all fields. The present studies will cement and further develop this fruitful scientific milieu in the cross-fields between clinical metabolism, cardiology, nuclear medicine and experimental clinical research. All methods described are well established, validated, used in many publications and known well known to the investigators. Similar study populations have been enrolled in previous trials; hence, inclusion of participants is feasible.

12 Budget and Economy

Operating costs – raw expenses:			
Utensils	:	40 x 1.250 DKK	= 50.000 DKK
Right heart catheters	:	40 x 1800 DKK	= 72.000 DKK
Electronic centralized CRF	:		= 25.000 DKK
Transportation of participants	:	80 x 50 x 3,75 DKK	= 15.000 DKK
Analysis of hormones and metabolites (40 analyses)	:		= 150.000 DKK
Purchase, purification and package of 3-OHB	:		= 90.000 DKK
Metabolism Studies	:		= 200.000 DKK
Subtotal	:		602.000 DKK
VIP salaries			
PhD salary 12 months	:	12 x 34.852DKK	= 418.228 DKK
PhD study fee 3 years			180.000 DKK
TAP salaries			
Technician 3 months	:		= 105.000 DKK
Grand total	**:**		**= 1.305.228 DKK**
Present funding			
Det Fri Forskningsråd	:		= 1.800.000 DKK
Novo Nordisk Fonden	:		= 1.600.000 DKK

The funds are administered by a research account that is subjected to public revision. No investigators have direct access research accounts. Professor Niels Møller is a member of the Independent Research Council on Medical Sciences. The investigators have no personal financial affiliations to any of the listed funds or companies.

13 Publications

Based on the proposed study we aim to publish 2 scientific papers. Positive, negative and inconclusive results will be published. Prior to publication, results will be presented at renowned international cardiovascular congresses. The papers will be submitted to international peer-reviewed journals with highest possible journal impact factor.

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