ChoCar trial; The role of gut microbiota in choline and carnitine metabolism on vascular inflammation in metabolic syndrome

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In this study we aim to investigate whether infusion of intestinal microbiota from lean (vegetarian) donors has differential effect on choline (d6-labeled choline) and carnitine (d3-labeled carnitine) metabolism and macrovascular inflammation (18F-...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Coronary artery disorders
Study type	Interventional

Summary

ID

NL-OMON39717

Source
ToetsingOnline

Brief title
ChoCar

Condition

- Coronary artery disorders
- Arteriosclerosis, stenosis, vascular insufficiency and necrosis

Synonym
Atherosclerosis, vessel calcification

Research involving
Human

Sponsors and support

Primary sponsor: Academisch Medisch Centrum
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: Choline and carnitine, Gut microbiota, Metabolic syndrome, Vascular inflammation

Outcome measures

Primary outcome

The primary endpoint concerns changes in postprandial choline and carnitine metabolism at baseline and 2 weeks after vegetarian microbial transplantation. We hypothesize that post-treatment changes are caused by altered gut microbiota composition. Thus fecal samples will be obtained for relating these changes.

Changes in gut microbiota composition

Morning stool samples will be collected at baseline, 1 and 2 weeks after start of the study to determine microbiota composition upon vegetarian microbial transplantation. Samples will be taken by collection on toilet paper or in a fecal container, to be directly frozen at -80 degrees C in the AMC. Fecal analysis will be done by HITChip array which is developed for exact and sensitive enumeration of bacterial population

Secondary outcome

Secondary parameters

We hypothesize that gut microbiota transplantation from lean vegetarian donors to obese recipients will lead to changes in choline and carnitine metabolism and subsequent decreases in arterial wall inflammation, in which both are

mediated by altered intestinal microbiota. To determine whether and to what extent this trait is transmissible via gut microbiota transplantation, we will also perform a CCCT and PET/CT-scan within the lean group. As such, differences in plasma TMA/TMAO between lean donors and their recipients two weeks after treatment can be determined.

Tertiary study parameters

Third endpoint are changes in macrovascular (aortic + carotid wall) inflammation (changes in TBR signal of aortic arch and carotid arteries) by obtaining 18F-FDG PET/CT-scan images as previously described in metabolic syndrome subjects, a procedure which is routine at the AMC department of Vascular Medicine

Fourth study parameters

Subcutaneous adipose tissue biopsies will be performed before and two weeks after treatment. They will be analysed for inflammatory markers to determine associations between gut microbiota composition, choline and carnitine metabolism and subcutaneous adipose tissue inflammation. This way we can correlate changes in inflammation in various tissues from source (intestine) to target (subcutaneous fat) and relate these changes to choline and carnitine metabolism.

Study description

Background summary

A role for gut microbiota in influencing metabolic pathways and as such playing a role in the etiology of metabolic disorders has been suggested . Whether and how the interplay between gut microbiota and metabolism can lead to cardiovascular diseases (CVD) is to be explored. Recently, the direct participation of gut microbiota in CVD pathogenesis was reported in the setting of a diet rich in phosphatidylcholine (PC) e.g. eggs, meat and other animal products, the major dietary source of choline. This study showed that gut microbiota-mediated catabolism of the choline moiety of PC produces trimethylamine (TMA), which in the liver is further metabolized to the proatherogenic species trimethylamine-N-oxide (TMAO), as well as the PC-metabolites betaine and choline. Plasma levels of TMAO, choline and betaine were associated with increased cardiovascular disease risk. Using orally gavaged choline- and PC-rich diets in mice and deuterium labeled PC (d9-PC) in humans, it was demonstrated that intestinal microbiota play an obligatory role in formation of TMA and consequently TMAO produced via induction of hepatic flavin-containing monooxygenases (FMO). Moreover, dietary supplementation with choline resulted in increased plasma TMAO as well as accelerated macrovascular inflammation in atherosclerosis-prone ApoE-knockout mice, underscoring causality. Interestingly, this pro-atherosclerotic phenomenon was not seen when mice were treated with broad spectrum antibiotics eliminating almost all gut microbiota. Importantly, supplementation of diet with the gut microbiota dependent metabolite TMAO accelerated atherosclerosis and macrovascular wall inflammation in the apolipoprotein E (ApoE)-knockout mice, consistent with TMAO playing a role in atherosclerosis.
Interestingly, the group of Hazen et al. also found that carnitine, abundant in red meat and that contains a trimethylamine moiety similar to choline, when metabolized also leads to increased TMAO-levels. Indeed in mice, oral carnitine supplementation caused increased atherosclerosis, an effect that was again completely diminished following suppression of intestinal microbiota with oral antibiotics. Moreover, antibiotic treatment followed by oral d3-labelled carnitine challenge was found to diminish plasma TMA and TMAO levels in mice and humans; a finding that was also observed in humans with a vegetarian (low red meat consumption) background (10). Apparently, proceeding dietary habits influence the capability of microbiota in the colon to produce TMA. As with choline, these results were confirmed by an observational study in humans, relating plasma carnitine levels to cardiovascular disease. Specifically, the association of high plasma carnitine levels with prevalent CVD and incident myocardial infarction (MI)/stroke and death risks were only observed with high TMAO levels as well * that is, subjects with high carnitine but low TMAO levels did not have increased cardiac risks. These results are consistent with TMAO playing a direct pro-atherogenic role, and intestinal microbiota playing an obligatory role in the formation of TMA (and thus TMAO) from trimethylamine-containing macronutrients such as PC, choline and carnitine.

Aforementioned studies did however not directly look at gut microbiota composition in relation to choline and carnitine metabolism. We recently showed beneficial effects of lean donor fecal transplantation on glucose metabolism in subjects with metabolic syndrome (MetS), which was mediated by specific changes in fecal bacterial species. Moreover, metabolic syndrome has recently been shown to be linked to increased aortic inflammation on PET-CT imaging. Thus, the aim of our study is to investigate the effect of allogenic (lean preferentially vegetarian donor) compared to autologous (own feces) microbial transplantation, derived from fecal samples, on gut microbiota composition and carnitine/choline metabolism as well as mascrovascular wall inflammation in obese males with metabolic syndrome.

Study objective

In this study we aim to investigate whether infusion of intestinal microbiota from lean (vegetarian) donors has differential effect on choline (d6-labeled choline) and carnitine (d3-labeled carnitine) metabolism and macrovascular inflammation (18F-FDG PET-CT scan) in obese subjects with metabolic syndrome and to associate changes in fecal gut microbiota after lean (vegetarian) donor microbial transplantation with above mentioned parameters.

Primary objective:
To determine changes in postprandial choline and carnitine metabolism at baseline and 2 weeks after lean donor microbial transplantation.

Secondary objective:
To compare choline and carnitine metabolism in the donors versus the recipients

Tertiary objective:
To evaluate the relation between changes in plasma choline and carnitine (metabolism) in relation to macrovascular inflammation

Fourth objective:
To evaluate associations between plasma choline and carnitine (metabolism) in relation to adipose tissue inflammation (subcutaneous fat biopsy).

Study design

This is a double blind single center randomized controlled trial.
Patients will be randomized by sealed envelopes to the following 2 treatment arms:
1. single allogenic (lean vegetrian donor) fecal transplantation (at baseline)
2. single autologous (own) fecal transplantation (at baseline)

Obese males are recruited via newspaper advertisements and screened for criteria of the MetS (3 or more out of 5 criteria according to updated NCEP

guidelines including at least increased fasting plasma glucose > 5.6 mmol/l) (13). Medication and supplement use (including vitamin/choline/carnitine supplements, energy drinks and carnitine-enriched soymilk) or a history of cardiovascular disease or cholescystectomy are exclusion criteria. When a potential subject is eligible, appointments will be made for all experiments to be completed during the study.

Donors are lean (BMI between 20-25 kg/m2) healthy males, preferentially vegetarian, and are also recruited via newspaper advertisements. As with the recipients, medication and use of food supplements (including vitamin/choline/carnitine supplements, energy drinks and carnitine-enriched soymilk) are exclusion criteria.

Both donor and recipient will be subjected to a postprandial (Nutridrink ingestion) oral choline/carnitine challenge. These tests can be done at the same time. The participants will be provided with a capsule of synthetic d3-carnitine and another capsule of d6-choline. The capsules will be taken at the same time simultaneously with Nutridink ingestion and plasma samples are taken at predefined time points to study plasma choline and carnitine metabolism. Moreover, an FDG PET-CT scan will be performed to assess level of macrovascular inflammation, as subjects with metabolic syndrome are characterized by an increased TBR signal as compared to control subjects.

Study program:
Week -1, Day 1 * 7 (lean vegetarian donors and metabolic syndrome subjects)
The preparation for week 0 starts at home with recording the dietary habits for the duration of one week within an online dietary booklet. Subjects are asked to adhere to their personal diet, without changing it throughout the study.

Week 0, Study day 1 (lean vegetarian donors and metabolic syndrome subjects)
At day 1, the first fat biopsy and dietary d6-choline/d3-carnitine challenge test (CCCT) are performed. Subjects and donors are asked to come to the clinical research unit after an overnight fast. After a subcutaneous fat biopsy and baseline blood sample, subjects are given a capsule containing 250mg of d6-choline and another capsule containing 250mg d3-carnitine to be ingested together with one bottle of Nutridrink (150cc). Hereafter serial blood samples are withdrawn for the duration of 6 hours. In addition, 24 hours of urine is collected and all participants are asked to obtain two tubes with feces from one fecal sample. At the end of the experiment, subjects are offered a meal to their choice.

Week 0, Study day 2 (lean vegetarian donors and metabolic syndrome subjects)
At Day 2, 18F-FDG PET-CT scan imaging of the aortic arch and carotid arteries is performed (12), followed by duodenal tube positioning via Coretrack. Afterwards bowel lavage will take place via the tube over 4 hours, which is followed by the actual treatment of either infusion of lean vegetarian donor-derived microbial solution (allogenic) or placebo (autologous feces). The

placement of the duodenal tube and the transplantation will only take place in the recipients. For the infusion of the fecal transplant material, both recipient and donor are asked to provide the study physician with a fresh fecal sample to enable double blind treatment.

Week 1, Study day 3 (metabolic syndrome subjects)
One week after the treatment, the first and only control visit is planned. Subjects status will be checked by a short interview, physical examination as well as a blood withdrawal for safety parameters. Also, subjects are asked to collect 24h urine in advance, which can be given to the study physician at the visit.

Week 1, day 1 * 7 (metabolic syndrome subjects)
The preparation for week 2 starts at home with recording the dietary habits for the duration of one week within an online dietary booklet. Subjects are asked to adhere to their personal diet, without changing it throughout the study.

Week 2, Study day 4 (metabolic syndrome subjects)
Two weeks after the treatment, the fat biopsy and CCCT is repeated, including the collection of two fecal samples and 24h urine.

Week 2, Study day 5 (metabolic syndrome subjects)
18F-FDG PET-CT scan imaging of the aortic arch en carotid arteries is performed. After the scan, subjects are allowed to leave the hospital and the study is completed.

In general, subjects are asked to be in a fasting state for all visits throughout the study, meaning they must have refrained from eating and drinking minimally 10 hours before start of the experiment.

Protocol lean vegetarian donors:
When eligible, donors are asked to also perform the fat biopsy and CCCT once, including obtaining 24hrs of urine and collection of 2 fecal samples as well as a PET-CT. This is to compare choline and carnitine metabolism between donors and recipients in relation to vascular and adipose tissue inflammatory status, both before and after treatment. As with recipients, donors are asked to write down their dietary habits the week before the CCCT is performed. Afterwards, donors will be randomly paired to one recipient. At the day of the transplantation, donors and metabolic syndrome subjects are both asked to provide a fecal sample to enable the treatment

Intervention

Patients will be treated with either allogenic or autologous microbial transplantion by duodenal tube after bowel lavage.

Both the donor and the subject will deliver a fresh feces sample (150-250g on

average) at the day of infusion (produced within 6 hours before use). After collection in a special container, feces will be stored at 4 degrees Celcius. Time of collection will be written down.
After having given a fecal sample to the study physician, subjects will be getting a duodenal tube placed via Coretrack device, which positioning will be checked via an abdominal X-ray. During this process and after randomization, one of both fecal samples will be mixed with 500cc saline solution (0.9% NaCl) until fully homogenised. Before mixing starts, a sample will be taken of the total amount of feces for later analysis. After mixing, the feces solution is poured through a sieve incorporated into a funnel to remove all debris and obtain a homogenous solution. The solution is poured into a 500cc sterile glass bottle. Thereafter, the bottle will be kept on refrigerator temperature (4 degrees Celcius) until the patients is finished with bowel lavage. When the intestines of the participant are completely cleared of fecal material, the treatment will take place. After treatment, subjects are offered a meal and are allowed to go home.

Study burden and risks

No adverse effects are expected in this study. However, subjects are asked for a time investment, frequent study visits, blood withdrawals as well as PET-CT scans and abdominal X-ray. In addition, they are subjected to behavioural changes, such as a dietary restriction, ingestion of choline and carnitine isotopes and the collection of faecal stool samples and urine. The isotopes do not confer any health risks.
The advantage for subjects is that their general health will be evaluated by physical examination and laboratory measurements. Based on their study results subjects will be given an advice with respect to their cardiovascular risk profile. In case abnormalities are found, subjects are referred to their general practitioner or to our outpatient clinic.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Subjects: Kaukasian, adult, obese males with BMI >30 kg/m2 and metabolic syndrome, including increased fasting glucose
Donors: healthy, adult, lean males (BMI 20-25 kg/m2) on a vegetarian diet

Exclusion criteria

Supplement use (including vitamin/choline/carnitine supplements, energy drinks and carnitine-enriched soymilk); a medical history of a cardiovascular event (myocardial infarction or stroke) or cholecystectomy; use of medication including antacids and oral antibiotics in the past three months; (expected) prolonged compromised immunity (e.g. due to recent cytotoxic chemotherapy or HIV-infection with a CD4 count < 240).

Study design

Design

Study type:	Interventional
Intervention model:	Parallel
Allocation:	Randomized controlled trial
Masking:	Double blinded (masking used)

Control:	Placebo
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	17-10-2013
Enrollment:	40
Type:	Actual

Ethics review

Approved WMO

Date:	04-09-2013
Application type:	First submission
Review commission:	METC Amsterdam UMC

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

ID: 24732
Source: Nationaal Trial Register
Title:

In other registers

Register	ID
CCMO	NL41928.018.12
OMON	NL-OMON24732