^{13}C-butyrate and ^{13}C-glucose breath testing to detect mesenteric ischemia, a proof of principal study in healthy volunteers (CMI breath test)

(June 2020)

PROTOCOL TITLE '^{13}C-butyrate and ^{13}C-glucose breath testing to detect mesenteric ischemia, a proof of principal study in healthy volunteers'

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Coordinating investigator/project leader	*L.G. Terlouw, MD* *Department of Gastroenterology & Hepatology, Erasmus MC University Medical Center, Rotterdam, the Netherlands* *l.terlouw@erasmusmc.nl*
Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder) ***<Multicenter research: per site>***	*Erasmus MC University Medical Center* *M.J. Bruno, MD, PhD* *Department of Gastroenterology & Hepatology,* *Erasmus MC University Medical Center, Rotterdam, the Netherlands* *m.bruno@erasmusmc.nl* *A. Moelker, MD, PhD* *Department of Radiology,* *Erasmus MC University Medical Center, Rotterdam, the Netherlands* *a.moelker@erasmusmc.nl* *M.P. Peppelenbosch, PhD* *Department of Gastroenterology & Hepatology,* *Erasmus MC University Medical Center, Rotterdam,*

	the Netherlands *m.peppelenbosch@erasmusmc.nl* *H.J.M. Verhagen, MD, PhD* *Department of vascular surgery,* *Erasmus MC University Medical Center, Rotterdam, the Netherlands* *h.verhagen@erasmusmc.nl* *T.M. Luider, PhD* *Department of Neurology,* *Erasmus MC University Medical Center, Rotterdam, the Netherlands* *t.luider@erasmusmc.nl* *Franciscus Gasthuis & Vlietland* *D. van Noord, MD, PhD* *Department of Gastroenterology & Hepatology,* *Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands* *d.leemreis@franciscus.nl*
Sponsor (in Dutch: verrichter/opdrachtgever)	*Erasmus University Medical Center Rotterdam*
Subsidising party	
Independent expert (s)	*A.C. de Vries, MD, PhD* *Department of Gastroenterology & Hepatology,* *Erasmus MC University Medical Center, Rotterdam,*

	the Netherlands *B.M. van Dalen, MD, PhD* *Department of Cardiology,* *Franciscus Gasthuis & Vlietland, Rotterdam,* *the Netherlands* *b.vandalen@franciscus.nl*
Laboratory sites <*if applicable*>	*Laboratorium MDL* *Erasmus MC University Medical Center,* *Wytemaweg 80,* *3015 CN Rotterdam, the Netherlands* *Da Vinci Laboratory Solutions B.V.* *Postbus 12103,* *3004 GC Rotterdam, the Netherlands*
Pharmacy <*if applicable*>	*Not applicable*

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Head of Department Gastroenterology & Hepatology: Prof. M.J. Bruno, MD, PhD Erasmus MC University Medical Centre, Rotterdam		30/6/2020
Principal Investigator: Prof. M.J. Bruno, MD, PhD Head of Gastroenterology & Hepatology department, Erasmus MC University Medical Centre, Rotterdam		30/6/2020

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
ATP	**Adenosine triphosphate**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CMI	**Chronic mesenteric ischemia**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**

VLS	**Visible light spectroscopy**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Chronic mesenteric ischemia (CMI) is an incapacitating disease that can progress to potentially fatal acute mesenteric ischemia. The yearly incidence of CMI is 9.2 per 100.000 and will increase due to the aging population and the rising prevalence of cardiovascular risk factors. A gold standard diagnostic test to diagnose CMI is currently lacking, causing both undertreatment and overtreatment of patients and thereby superfluous healthcare expenses. Since oxygen is needed to absorb and metabolise butyrate and glucose in the enterocyte, a ^{13}C-butyrate and ^{13}C -glucose breath test could theoretically quantify mucosal oxygen content and thereby identify patients with CMI.

Objective: To explore the possibility of detecting mesenteric ischemia by ^{13}C-butyrate and ^{13}C -glucose breath testing by comparing timing and concentration of the peak of expired $^{13}CO_2$.

Study design: randomised intervention study

Study population: healthy human volunteers, >18 old

Intervention (if applicable): The intervention group will perform a standardized bicycle ergometry exercise test with a duration of 30 minutes. The aim of the exercise test is to induce temporary mesenteric ischemia.

Main study parameters/endpoints: To explore the possibility of detecting mesenteric ischemia by ^{13}C-butyrate and ^{13}C -glucose breath testing by comparing timing and concentration of the peak of expired $^{13}CO_2$.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: All participating volunteers will perform 2 breath tests at baseline, 0.5, 1, 1.25 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5 and 4 hours after ingestion of ^{13}C-butyrate or ^{13}C-glucose. A peripheral intravenous cannula will be inserted in patients in the intervention group, in order to obtain a serum blood sample at baseline and after the exercise test. The cannula will also be used to obtain blood for the lactate measurements during the exercise test. Participating volunteers will not experience any benefits of participation. No adverse events are expected and risks associated with participation are deemed very low to non-existent. Insertion of the peripheral intravenous cannula can result in a hematoma.

1. INTRODUCTION AND RATIONALE

Chronic mesenteric ischemia (CMI) is an invalidating disease, causing severe complaints of post-prandial pain, food fear and weight loss(1, 2). When left untreated CMI has a substantial risk of bowel infarction in 26-67% of patients, resulting in high mortality and morbidity (3, 4). Diagnosis of CMI remains difficult, since no gold standard diagnostic test exists. The gold standard diagnosis described in current literature is relief of symptoms after endovascular or surgical revascularisation. In order to take treatment decisions a consensus diagnosis is used. Consensus is reached during a multidisciplinary meeting attended by gastroenterologists, vascular surgeons and interventional radiologists. Consensus is based on history, presence of mesenteric artery stenosis on abdominal imaging and in dedicated centres a functional test, such as visible light spectroscopy (VLS) and tonometry. In patients with a consensus diagnosis of CMI based on a stenosis of a single mesenteric artery, treatment is successful in 73% of patients(5). In the remaining 27% not benefitting from treatment, CMI was never the cause of the patients symptoms.

A more accurate functional test detecting intestinal ischemia is of great value to improve the used consensus diagnosis. Current functional tests have several downsides and are therefore not widely used. VLS, a technique measuring mucosal saturations, is limited by a specificity of 60%, causing a substantial amount of false positive test results(6). Tonometry, a technique measuring luminal carbon dioxide concentrations in stomach and jejunum, has a better specificity of 92%(6). Yet this test will cease to exist in a few years, since tonometry equipment is no longer produced. Stressing the urgency of development of a new and improved functional test.

Breath tests are accurate, simple and widely available tests for diseases, such as helicobacter pylori infection and small intestinal bacterial overgrowth(7, 8). Breath tests using glucose and butyrate could possibly be used to diagnose CMI. Butyrate is metabolized by the intestinal epithelium(9). Metabolism of butyrate is oxygen dependent, since oxidation is one of the steps during the production of adenosine triphosphate (ATP). ^{14}C-butyrate can be used to measure the oxidation rate of butyrate(10). During the oxidation process the ^{14}C atom is transferred to an oxygen molecule, producing ^{14}C-carbon dioxide. The labelled carbon dioxide molecule is exhaled and can be measured in the air exhaled by the patient, thereby quantifying the presence of mucosal oxygen.

Administration of deuterated glucose (^{13}C-glucose) is a second potential method to measure mucosal oxygen content. Transport of glucose from the lumen into the epithelial cell is driven by an active process consuming ATP and thus oxygen(11). The transport of glucose is driven by a sodium gradient. At the basolateral side of the cell, Na^+/K^+-ATPase extrudes sodium, thereby creating a low sodium concentration within the cell. At the luminal side a Na^+/glucose cotransporter, driven by the low intracellular sodium concentration, transports sodium and glucose into the cell. Further transport of glucose into the mesenteric circulation occurs by diffusion facilitated by passive glucose transporters. When ^{13}C-glucose is metabolized the ^{13}C atom is transferred to an oxygen molecule, producing ^{13}C-carbon dioxide. The labelled carbon dioxide molecule is exhaled and can be measured in the air exhaled by the patient.

In subjects with low mucosal oxygen concentrations, the peak in exhaled $^{13}CO_2$ of butyrate and glucose is expected to be delayed and lower compared to control subjects with normal mucosal oxygen concentrations. Thereby creating a functional test measuring mucosal oxygen content, which could be of great value in diagnosing CMI.

2. OBJECTIVES

Primary Objective:

To explore the possibility of detecting mesenteric ischemia by ^{13}C-butyrate and ^{13}C-glucose breath testing by comparing timing and concentration of the peak of expired $^{13}CO_2$.

3. STUDY DESIGN

This study is a multi-center randomized interventional proof of principal study, exploring the possibility of quantifying mucosal oxygen content by ^{13}C-butyrate and ^{13}C-glucose breath testing.

Inclusion

The study is conducted in healthy volunteers without gastrointestinal complaints and unremarkable medical history. Volunteers are recruited by distribution of patient information folders, containing study information and the investigators contact information, at the gastroenterology and Hepatology outpatient clinics of Erasmus MC University Medical Center and Franciscus Gasthuis & Vlietland. When volunteers contact the investigator the study outline is discussed and inclusion and exclusion criteria are checked. Volunteers are asked whether they have a medical history or are currently experiencing any complaints, they do not undergo additional tests or check-ups to ensure they are healthy. When a healthy volunteer decides to participate in the study an Informed Consent Form will be signed.

Control

Two different control groups both consisting of 5 volunteers will be used during the study. The first group will receive ^{13}C-butyrate and the second group ^{13}C-glucose. The control groups will perform the breath tests without performing any physical exercise.

Intervention

Two different intervention groups both consisting of 5 volunteers will be used during the study. The first group will receive ^{13}C-butyrate, the second group ^{13}C-glucose. All intervention groups will be performing a standardized bicycle ergometer exercise test with a total duration

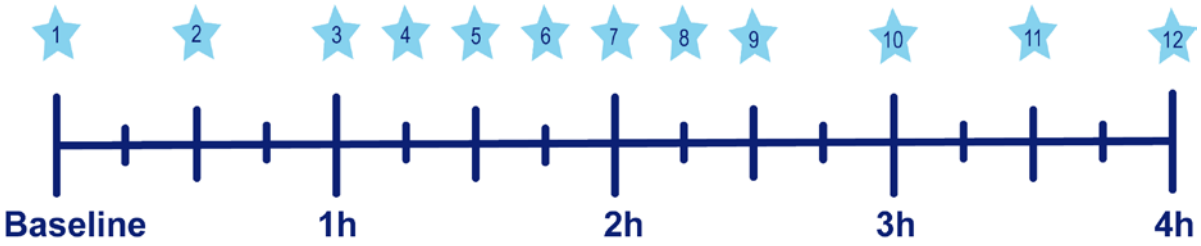
of 30 minutes(12). The exercise test has been proven to elicit mesenteric ischemia in athletes. Previous studies suggest an additional 10 minute period of splanchnic hypo perfusion after discontinuation of exercise(13, 14).
The exercise test consist of 3 phases.

- Phase 1: The first 10 minutes of exercise are used to gradually increase the workload until submaximal exercise intensity is reached. Submaximal exercise is defined as lactate between 3 and 5.5 mmol/L. Lactate measurements are performed every 2 minutes, using a rapid lactate measurement kit.
- Phase 2: From minute 10 until 20 submaximal exercise intensity is maintained by adjusting the workload based on lactate measurements. The lactate measurements are performed every 3 minutes.
- Phase 3: Minute 20 until 30 are used to reach maximal exercise intensity. Every 3 minutes the workload is increased by 10% of the submaximal workload until exhaustion. Lactate measurements will be continued with 3 minute intervals.

Randomisation
Allocation to the ^{13}C-butyrate or ^{13}C-glucose group and allocation to the control or intervention group will be performed by randomisation. Blinding of the healthy volunteers will not be possible, since the intervention group has to perform an exercise test.

Breath test
Both intervention and control group will perform breath tests at the following time points baseline, 0.5, 1, 1.25 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5 and 4 hours after ingestion of ^{13}C-butyrate or ^{13}C-glucose (figure 1). A breath sample is obtained by blowing a single breath of air through a straw into a test tube. Two breath samples are collected at all mentioned time points. The decision to perform breath testing for a total of 4 hours was based on a study that determined $^{14}CO_2$ elimination patterns after instillation of butyrate in human caecum and a study describing $^{13}CO_2$ elimination patterns in healthy volunteers(10, 15). The tracer could be measured in exhaled air at the first time point, which was 30 minutes after instillation. A peak in expired $^{14}CO_2$ was measured after 2 hours. Four hours after instillation approximately 55% of the total $^{14}CO_2$ dose had been expired, 24 hours after instillation 70% of the instilled dose had been expired. Since we are interested in the peak of expired $^{13}CO_2$ a measurement period of 4 hours seems sufficient, even in the intervention group, in whom a delayed or lower peak is expected. The frequency of breath testing is intensified between 1 and 2.5 hours after ingestion, in order to detect the timing of the $^{13}CO_2$ peak more precisely.

Figure 1. timeline collection of breath samples.



Dosages

All volunteers will be instructed to take their last meal 6 hours before start of the breath test.

Volunteers in the ^{13}C-butyrate group receive an oral dose of 0.03 µmol ^{13}C-butyrate(10).

Volunteers in the ^{13}C-glucose group receive an oral dose of 20 mmol ^{13}C-glucose(16).

Follow-up

There is no follow-up of healthy volunteers.

4. STUDY POPULATION

a. Population (base)

Healthy volunteers with no gastrointestinal complains and unremarkable medical history will be asked to participate in our study through information folders.

b. Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- ≥18 years of age
- Experience with cycling
- Signed informed consent
- Unremarkable medical history (no gastrointestinal diseases, no cardiac or pulmonary diseases)
- No gastrointestinal complaints

c. Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Unable to sign informed consent
- Pregnancy

d. Sample size calculation

This study is a proof of principal study, no previous studies exist on this topic, therefore a sample size calculation could not be performed. We have chosen to include a total of 20 healthy volunteers in order to be able to compare 5 control subjects and 5 intervention

subjects for each breath test. In case the number of 5 subjects per group is insufficient to detect a statistical difference, the generated data can be used to perform a sample size calculation for a second study.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Intervention

Two different intervention groups both consisting of 5 volunteers will be used during the study. The first group will receive ^{13}C-butyrate and the second group ^{13}C-glucose. Both intervention groups will be performing a standardized bicycle ergometer exercise test with a total duration of 30 minutes(12). The exercise test has been proven to elicit mesenteric ischemia in athletes. Previous studies suggest an additional 10 minute period of splanchnic hypo perfusion after discontinuation of exercise(13, 14).

The exercise test consist of 3 phases.

- Phase 1: The first 10 minutes of exercise are used to gradually increase the workload until submaximal exercise intensity is reached. Submaximal exercise is defined as lactate between 3 and 5.5 mmol/L. Lactate measurements are performed every 2 minutes, using a rapid lactate measurement kit.
- Phase 2: From minute 10 until 20 submaximal exercise intensity is maintained by adjusting the workload based on lactate measurements. The lactate measurements are performed every 3 minutes.
- Phase 3: Minute 20 until 30 are used to reach maximal exercise intensity. Every 3 minutes the workload is increased by 10% of the submaximal workload until exhaustion. Lactate measurements will be continued with 3 minute intervals.

Control

Two different control groups both consisting of 5 volunteers will be used during the study. The first group will receive ^{13}C-butyrate and the second group ^{13}C-glucose. The control groups will perform the breath tests without performing any physical exercise.

Dosages

All volunteers will be instructed to take their last meal 6 hours before start of the breath test. Volunteers in the ^{13}C-butyrate group receive an oral dose of 0.03 µmol ^{13}C-butyrate(10). Volunteers in the ^{13}C-glucose group receive an oral dose of 20 mmol ^{13}C-glucose(16).

5.2 Use of co-intervention (if applicable)

Not applicable

5.3 Escape medication (if applicable)

Not applicable

6 INVESTIGATIONAL PRODUCT

Not applicable

6.1 Name and description of investigational product(s)

Not applicable

6.2 Summary of findings from non-clinical studies

Not applicable

6.3 Summary of findings from clinical studies

Not applicable

6.4 Summary of known and potential risks and benefits

Not applicable

6.5 Description and justification of route of administration and dosage

Not applicable

6.6 Dosages, dosage modifications and method of administration

Not applicable

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable

6.8 Drug accountability

Not applicable

7 NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

^{13}C-butyrate is a stable isotope used as a tracer molecule.
https://www.sigmaaldrich.com/catalog/product/aldrich/488380?lang=en&region=NL

D-Glucose-1-^{13}C is a stable isotope used as a tracer molecule.

https://www.sigmaaldrich.com/catalog/product/aldrich/297046?lang=en&region=NL

7.2 Summary of findings from non-clinical studies

^{13}C-butyrate

We refer to the safety information statement on the website of Sigma-Aldrich
https://www.sigmaaldrich.com/catalog/product/aldrich/488380?lang=en&region=NL

D-Glucose-1-^{13}C

We refer to the safety information statement on the website of Sigma-Aldrich
https://www.sigmaaldrich.com/catalog/product/aldrich/297046?lang=en&region=NL

7.3 Summary of findings from clinical studies

^{13}C-butyrate

A study including 12 healthy volunteers and 12 Crohn's disease patients showed that ^{13}C-butyrate absorption metabolism can be measured by $^{13}CO_2$ breath testing(17). They detected $^{13}CO_2$ at 30 minutes after oral administration of ^{13}C-butyrate, with a peak $^{13}CO_2$ concentration at 1 hour after administration. A study showed similar results and timing of expired $^{14}CO_2$ concentrations after instillation of ^{14}C-butyrate in the cecum(10).

D-Glucose-1-^{13}C

A study including 8 healthy highly trained volunteers examined the oxidation of ^{13}C-glucose during exercise(16). This study showed that $^{13}CO_2$ could be measured reliably, in expired air, after administration of a ^{13}C-glucose dose of 0.05g/kg bodyweight.

7.4 Summary of known and potential risks and benefits

^{13}C-butyrate

We refer to the safety information statement on the website of Sigma-Aldrich
https://www.sigmaaldrich.com/catalog/product/aldrich/488380?lang=en&region=NL

D-Glucose-1-^{13}C

We refer to the safety information statement on the website of Sigma-Aldrich
https://www.sigmaaldrich.com/catalog/product/aldrich/297046?lang=en&region=NL

7.5 Description and justification of route of administration and dosage

^{13}C-butyrate

A study performing $^{14}CO_2$ breath testing after instillation of ^{14}C-butyrate in the cecum showed that a tracer dose of 0.03 µmol was sufficient and could be detected in expired air(10).

D-Glucose-1-^{13}C

A study performing $^{13}CO_2$ breath testing during exercise showed that $^{13}CO_2$ could be measured reliably after administering an oral dose of 0.05g/kg bodyweight ^{13}C-glucose(16). This dosage equals approximately 20mmol of glucose, when using a bodyweight of 70kg.

7.6 Dosages, dosage modifications and method of administration

^{13}C-butyrate

A study performing $^{14}CO_2$ breath testing after instillation of ^{14}C-butyrate in the cecum showed that a tracer dose of 0.03 µmol was sufficient and could be detected in expired air(10).

D-Glucose-1-^{13}C

A study performing $^{13}CO_2$ breath testing during exercise showed that $^{13}CO_2$ could be measured reliably after administering 0.05g/kg bodyweight ^{13}C-glucose(16). This dosage equals approximately 20mmol of glucose, when using a bodyweight of 70kg.

Both ^{13}C-butyrate and D-Glucose-1-^{13}C are administered orally, since metabolism and absorption by the enterocytes are the processes that are of interest.

7.7 Preparation and labelling of Non Investigational Medicinal Product

Preparation and labelling of the non-investigational medicinal products will be done according to the relevant GMP guidelines by licensed staff.

7.8 Drug accountability

^{13}C-butyrate and D-Glucose-1-^{13}C solutions will be prepared at the Gastroenterology & Hepatology department.

8 METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

To explore the possibility of detecting mesenteric ischemia by ^{13}C-butyrate and ^{13}C-glucose breath testing by comparing timing and concentration of the peak of expired $^{13}CO_2$.

8.1.2 Secondary study parameters/endpoints (if applicable)

Not applicable

8.1.3 Other study parameters (if applicable)

Not applicable

8.2 Randomisation, blinding and treatment allocation

Participating volunteers will not be blinded. Randomization will be performed into four groups of five volunteers.

Randomisation will be performed through a random list generated by an online randomisation tool and is executed by a person not actively involved in the study. The randomisation tool can be found at https://www.sealedenvelope.com/simple-randomiser/v1/lists. The used seed will be noted and saved, the treatment groups are: BC, BI, GC, GI. Block sizes will be 5 blocks of 4, resulting in a randomized list of 20 subjects (5 per group). The created list will be saved and used to fill 20 envelopes numbered 1-20. Each envelope will contain the treatment group as specified in the randomly generated list. Envelope 1 will be for the first subject enrolled in the study, envelope 2 for the second subject enrolled in the study, etc.

8.3 Study procedures

Inclusion

The study is conducted in healthy volunteers without gastrointestinal complaints and unremarkable medical history. Volunteers are recruited by distribution of patient information folders, containing study information and the investigators contact information, at the gastroenterology and Hepatology outpatient clinics of Erasmus MC University Medical Center and Franciscus Gasthuis & Vlietland. When volunteers contact the investigator the study outline is discussed and inclusion and exclusion criteria are checked. Volunteers are asked whether they have a medical history or are currently experiencing any complaints, they do not undergo additional tests or check-ups to ensure they are healthy. When a healthy volunteer decides to participate in the study an Informed Consent Form will be signed.
Volunteers will be randomized and assigned to one of four study groups.

Control

Two different control groups both consisting of 5 volunteers will be used during the study. The first group will receive ^{13}C-butyrate and the second group ^{13}C-glucose. The control groups will perform the breath tests without performing any physical exercise.

Intervention

Two different intervention groups both consisting of 5 volunteers will be used during the study. The first group will receive ^{13}C-butyrate and the second group ^{13}C-glucose. All intervention groups will be performing a standardized bicycle ergometer exercise test with a total duration of 30 minutes(12). The exercise test has been proven to elicit mesenteric ischemia in athletes. Previous studies suggest an additional 10 minute period of splanchnic hypo perfusion after discontinuation of exercise(13, 14).

The exercise test consist of 3 phases.

- Phase 1: The first 10 minutes of exercise are used to gradually increase the workload until submaximal exercise intensity is reached. Submaximal exercise is defined as lactate between 3 and 5.5 mmol/L. Lactate measurements are performed every 2 minutes, using a rapid lactate measurement kit.
- Phase 2: From minute 10 until 20 submaximal exercise intensity is maintained by adjusting the workload based on lactate measurements. The lactate measurements are performed every 3 minutes.
- Phase 3: Minute 20 until 30 are used to reach maximal exercise intensity. Every 3 minutes the workload is increased by 10% of the submaximal workload until exhaustion. Lactate measurements will be continued with 3 minute intervals.

All volunteers will be instructed to take their last meal 6 hours before start of the breath test. Volunteers in the ^{13}C-butyrate group receive an oral dose of 0.03 µmol ^{13}C-butyrate(10). Volunteers in the ^{13}C-glucose group receive an oral dose 20 mmol ^{13}C-glucose.

Randomisation

Allocation to the ^{13}C-butyrate and ^{13}C-glucose group and allocation to the control or intervention group will be performed by randomisation. Blinding of the healthy volunteers will not be possible, since the intervention group has to perform an exercise test.

Breath test

Both intervention and control group will perform breath tests at the following time points baseline, 0.5, 1, 1.25 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5 and 4 hours after ingestion of ^{13}C-butyrate

or ^{13}C-glucose. Two breath samples are collected at all mentioned time points. The decision to perform breath testing for a total of 4 hours was based on a study that determined $^{14}CO_2$ elimination patterns after instillation of butyrate in human caecum and a study describing $^{13}CO_2$ elimination patterns in healthy volunteers(10, 15). The tracer could be measured in exhaled air at the first time point, which was 30 minutes after instillation. A peak in expired $^{14}CO_2$ was measured after 2 hours. Four hours after instillation approximately 55% of the total $^{14}CO_2$ dose had been expired, 24 hours after instillation 70% of the instilled dose had been expired. Since we are interested in the peak of expired $^{13}CO_2$ a measurement period of 4 hours seems sufficient, even in the intervention group, in whom a delayed or lower peak is expected. The frequency of breath testing is intensified between 1 and 2.5 hours after ingestion, in order to detect the timing of the $^{13}CO_2$ peak more precisely.

Follow-up
There is no follow-up of healthy volunteers.

Study procedures	**Control group**	**Intervention group**
Oral dose of either 0.03 µmol ^{13}C-butyrate or 20 mmol ^{13}C-glucose	Yes	Yes
Insertion of venous canule (measurement of lactate, 2 serum samples: 1 at baseline and 1 after the exercise test)	No	Yes
Bicycle ergometer exercise test	No	Yes
Collection of 2 breath samples at baseline, 0.5, 1, 1.25 1.5, 1.75, 2, 2.25, 2.5, 3, 3.5 and 4 hours after ingestion of ^{13}C-butyrate or ^{13}C-glucose	Yes	Yes

Analysis of breath samples
Measurement of $^{13}CO_2$ will be performed using mass spectrometry (IDMicro Breath, Compact Science Systems, Newcastle-Under-Lyme, United Kingdom). The mass spectrometry procedure is equal to the procedures used to measure $^{13}CO_2$ in testing for Helicobacter Pylori, which is current clinical practice.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal (if applicable)

Not applicable

8.5 Replacement of individual subjects after withdrawal

Inclusion will end after inclusion of 5 volunteers in each of the four groups.

8.6 Follow-up of subjects withdrawn from treatment

Follow-up is not performed.

8.7 Premature termination of the study

The study will be terminated prematurely when participation is associated with serious health risks, which are not expected.

9 SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as an undesirable experience occurring to a subject during the study, related to the study. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

9.3 Annual safety report

Not applicable

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

Not applicable

10 STATISTICAL ANALYSIS

Baseline characteristics of the intervention and control group will be shown and compared. Categorical data is compared by chi-square or Fisher exact testing. Numerical data is shown as mean and standard deviation or median and interquartile range. Comparison of the numerical data of intervention and control group will be performed by the independent sample t-test or the Mann-Whitney-U test.

10.1 Primary study parameter(s)

The height of the $^{13}CO_2$ peak of the intervention and control groups will be compared by the independent sample t-test or Mann-Whitney-U test. Repeated measurement correction will be performed with the Bonferroni method. The area under the $^{13}CO_2$ curves will be compared by the independent sample t-test or Mann-Whitney-U test.

10.2 Secondary study parameter(s)

Not applicable

10.3 Other study parameters

Not applicable

10.4 Interim analysis (if applicable)

Not applicable

11 ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be performed according to the principles of the Declaration of Helsinki, of Good Clinical Practice, and the applicable laws and regulations of the Netherlands, including but not limited to the Medical Research Involving Human Subjects Act (WMO) and the Personal Data Protection Act.

11.2 Recruitment and consent

Volunteers are recruited by distribution of patient information folders, containing study information and the investigators contact information, at the gastroenterology and Hepatology outpatient clinics of Erasmus MC University Medical Center and Franciscus Gasthuis & Vlietland. When volunteers contact the investigator the study outline is discussed and inclusion and exclusion criteria are checked. Volunteers are asked whether they have a medical history or are currently experiencing any complaints, they do not undergo additional tests or check-ups to ensure they are healthy. When a healthy volunteer decides to participate in the study an Informed Consent Form will be signed.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable

11.4 Benefits and risks assessment, group relatedness

Participation in this study will not be beneficial for the healthy volunteers. However, this study will provide a valuable insight into the feasibility of ^{13}C-butyrate and ^{13}C-glucose breath testing. This proof-of-principal study is the first step in the development of a more accurate and less cumbersome and less invasive functional test for the detection of CMI.

There are no known side-effects of the administration of ^{13}C-glucose. Administration of high concentrations of ^{13}C-butyrate could result in diarrhoea, however tracer dosages are used in this study.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives (if applicable)

Not applicable

12 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

The handling of personal data will be compatible with the Code of Good Behaviour (Code goed gedrag) and the Dutch Personal Data Protection Act (Wet Bescherming Persoonsgegevens). The collected data will be recorded in an electronic database, which contains all relevant clinical data of the volunteers. This database will be provided with a password, which is only available for the investigators. The database will be anonymized by replacing all names of volunteers by a code and will be used for statistical analysis. Only these codes will be used for references in reports and publications about this investigation. Volunteers will be asked for their permission to store the material for 15 years and that it could be used for scientific research in the future with the same goal.

12.2 Monitoring and Quality Assurance

Not applicable

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient’s last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

Results of the study will be published in an international journal. There are no limitations or restrictions for publication mention the arrangements made between the sponsor and the investigator concerning the public disclosure and publication.

STRUCTURED RISK ANALYSIS

12.7 Potential issues of concern

a. Level of knowledge about mechanism of action
Both ^{13}C-butyrate and ^{13}C-glucose are stable isotopes

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism
^{13}C-butyrate and ^{13}C-glucose have been administered in human subjects(10). Adverse events were not reported.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?
Not applicable

d. Selectivity of the mechanism to target tissue in animals and/or human beings
Both ^{13}C-butyrate and ^{13}C-glucose can be metabolized in cells other than enterocytes, yet in this case the timing of the peak is expected to be delayed, since absorption and transportation through the bloodstream are required.

e. Analysis of potential effect
A study performing $^{14}CO_2$ breath testing after instillation of ^{14}C-butyrate in the cecum showed that a tracer dose of 0.03 µmol was sufficient and could be detected in expired air(10). A study has shown that a ^{13}C-glucose dose of 0.05 gr/kg bodyweight (approximately 3.5gr in an individual of 70 kg) could be detected in expired air. The effects of exercise induced mesenteric ischemia on the breath test patterns have not been studied.

f. Pharmacokinetic considerations
Not applicable

g. Study population
Heathy volunteers have been used in previous studies administering ^{13}C-butyrate and ^{13}C-glucose.

h. Interaction with other products
No drug interactions are expected, since ^{13}C-butyrate and ^{13}C-glucose are metabolized by the enterocytes.

i. Predictability of effect

A study that determined $^{14}CO_2$ elimination patterns after instillation of butyrate in human caecum and a study described $^{13}CO_2$ elimination patterns in healthy volunteers(10, 15). The tracer could be measured in exhaled air at the first time point, which was 30 minutes after instillation. A peak in expired $^{14}CO_2$ was measured after 2 hours. Four hours after instillation approximately 55% of the total $^{14}CO_2$ dose had been expired, 24 hours after instillation 70% of the instilled dose had been expired. Since we are interested in the peak of expired $^{13}CO_2$ a measurement period of 4 hours seems sufficient, even in the intervention group, in whom a delayed or lower peak is expected.

j. Can effects be managed?

No effects are expected, but when unexpected effects do occur the exercise test can be stopped.

12.8 Synthesis

Not applicable

13 REFERENCES

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