Breath Metabolomics of Placebo Effects, a Pilot Study (BMPE)

Study Type:	Other Clinical Trial according to ClinO, Chapter 4
Risk Categorisation:	Risk category A according to ClinO, Art. 61
Study Registrations:	BASEC: 2021-01132 SNCTP: 000004536 clinicaltrials.gov: NCT04956718
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Investigated Intervention:	Breath metabolomics of placebo effects via cold pressor test
Protocol ID	BMPE
Version and Date:	Version 1.2 (dated 15/09/2021)

PROTOCOL SIGNATURE FORM

Study Title Breath Metabolomics of Placebo Effects, a Pilot Study

The Sponsor-Investigator has approved the protocol version 1.2 (dated 15/09/2021) and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, and ICH-GCP guidelines as well as the local legally applicable requirements.

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Date: 15.09.2021

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Date: 15.09.2021

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GLOSSARY OF ABBREVATIONS

AE	*Adverse Event*
ASR/DSUR	*Annual Safety Repot / Development Safety Report*
BASEC	*Business Administration System for Ethical Committees*
CNS	*Central Nervous System*
CRF	*Case Report Form*
CPT	*Cold Pressor Test*
CTCAE	*Common Terminology Criteria for Adverse Events*
FADP	*Federal Act on Data Protection (in German: DSG, in French: LPD, in Italian: LPD)*
eCRF	*electronic Case Report Form*
fMRI	*Functional Magnetic Resonance Imaging*
FOPH	*Federal Office of Public Health*
GC	*General Consent*
GCP	*Good Clinical Practice*
HRA	*Human Research Act (in German: HFG, in French: LRH, in Italian: LRUm)*
ICH	*International Conference on Harmonisation*
IC	*Informed Consent*
M/Z	*Mass to Charge Ratio*
SESI-MS	*Secondary Electrospray Ionization-Mass Spectrometry*
UKBB	*University Children's Hospital Basel*
ClinO	*Ordinance on Clinical Trials in Human Research (in German: KlinV, in French: OClin, in Italian: OSRUm)*
SAE	*Serious Adverse Event*

1 BACKGROUND AND RATIONALE

1.1 Background

Placebo is key in modern clinical practice, whereby it is widely used as control experiment in multiple trials. The neurobiological basis of placebo analgesia has been studied for over 40 years, since Levine at al. showed that the opiate antagonist naloxone could annul placebo analgesia after wisdom tooth extraction (Levine, 1978). More recent studies suggest that placebos can alter the experience of pain. For example, a recent study using functional magnetic resonance imaging (fMRI), found that placebo analgesia was related to decreased brain activity in pain-sensitive brain region (Wager TD, 2004). At the genomic level, it has also been shown that placebo analgesia might be objectively quantifiable (Hall KT, 2015). The literature addressing the question of placebo effects at the metabolomics level is very limited. However, the few studies addressing this, suggest a measurable, but not overwhelming effect (Kaddurah-Daouk R. B., 2013) (Kaddurah-Daouk R. B., 2011).

1.2 Rationale

Understanding the biological mechanisms of the placebo effects have a great and overarching impact into the broad field of clinical interventions. Amongst others, placebo effects tend to obscure efficacy of drugs. Any new tool to reduce this unwanted data variability, would benefit clinical trials as well as clinical practice and patient care. Furthermore, it may also reduce patient's exposure to drugs they might not need, henceforward reducing drug side-effects in overall population. Defining who might respond to placebo and how this happens biologically is of great importance as those who are very likely to respond to placebo could be excluded from clinical trials to increase the chances for seeing a drug-specific effect.

The literature suggests that placebo effects induce specific metabolic alterations, however the body of evidence is rather limited. This pilot study will provide additional insights into this very limited studied subject by addressing the question of whether placebo effects induces any measurable changes at the metabolic level. Because exhaled breath contains relevant metabolic information easily accessible, we will exploit this feature by interrogating the breath metabolome before and after the administration of a placebo in an established experimental pain procedure in healthy subjects.

2 STUDY OBJECTIVES AND DESIGN

3.1 Hypothesis and primary objective

Our hypothesis is that placebo effects exists, and that they modulate certain metabolic pathways. We will test this hypothesis by interrogating the exhaled breath metabolome while using an objective method of measurement for placebo effects in pain analgesia

(i.e. Cold Pressor Test; CPT). CPT reactions can be measured objectively by blood pressure spikes as well as changes in continuous heart rate monitoring. Those values have proven to be reliable in numerous studies and the model is ideal for measuring reduction of pain by various pharmaceuticals (Modir, 2010).

3.2 Primary and secondary endpoints

Primary endpoint

The variable of primary interest is the variation of signal intensity of mass-to-charge ratio (m/z) of exhaled metabolites across the conducted CPT pain analgesia-placebo effects trial.

Secondary endpoints

Differentially exhaled metabolites across the pain vs. no-pain measurements.
Exhaled metabolites for differences between placebo responders and non-responders.
Exhaled metabolites association with blood pressure and heart rate.

3.3 Study design

Single center; national, implemented at the University Children's Hospital Basel (UKBB).
Randomized Crossover design; Pilot Study.

3.4. Study intervention

Visit A (CPT, 60 min)

The participant receives an explanation how this study will work in terms of exhalation maneuvers, medication and CPT and signs informed consent (IC) 1 if this is the first visit. The participant will then be connected to a patient monitor for continuous tracking of heart rate and blood pressure. An exhalation set typically contains six replicate exhalations in positive and negative ion mode. The method is highly standardized, as described in (Singh, Tancev et al. 2019), whereby exhalation maneuvers are controlled by a mass flow controller and guided by CO_2 exhalation. Typical coefficients of variation for such replicate measurements are in the order of 7%. In total, this process takes around seven minutes. First, two baseline exhalations are performed. Then, the CPT is performed according to the 'CPT Guide' (for details, see below Figure 3). This takes around 5min. Directly after the CPT, the proband executes first post-pain exhalations set. Probands then rest 15min and executes the second post-pain exhalations set. In case this is the second visit, probands receive 100CHF. At all times, a medical doctor will be available inside the UKBB premises in case the participant requires medical assistance.

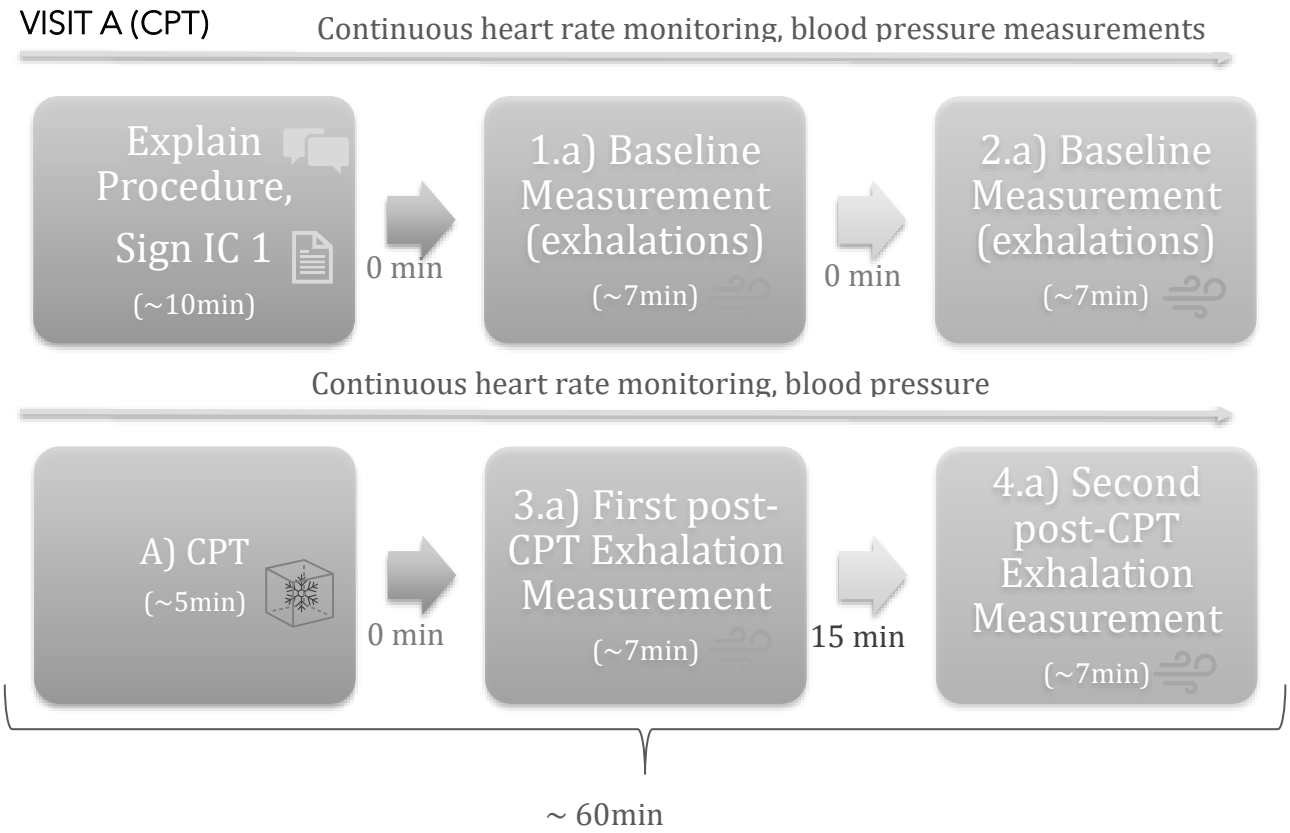

Figure 1. Schematic flowchart and process description of the study visit A

Visit B (CPT+Placebo, 60 min)

The participant receives an explanation how this study will work in terms of exhalation maneuvers, CPT and 'medication' (Placebo NaCl Nasal spray) and signs IC 1 if this is the first visit. The participant will then be connected to a patient monitor for continuous tracking of heart rate and blood pressure. The procedure is the same as in Visit A with the exception that participants will be told and read about the 'pain medication' (Appendix 3, the goal here is to enhance Placebo effects, Appendix 7: script of what the participants will be told about the medication), and that thereafter participants will self-administer a nasal spray, which is in fact a placebo nasal spray containing NaCl solution. Then, the CPT according to the 'CPT Guide' is performed (for details, see below). This takes around 5min. Directly after the CPT, the proband executes first post-pain exhalations set. Probands then rest 15min and executes the second post-pain exhalations set. In case this is the second visit, probands are debriefed and sign the IC 2. At all times, a medical doctor will be available in the UKBB premises in case the participant requires medical assistance.

VISIT B (CPT+ PLACEBO)

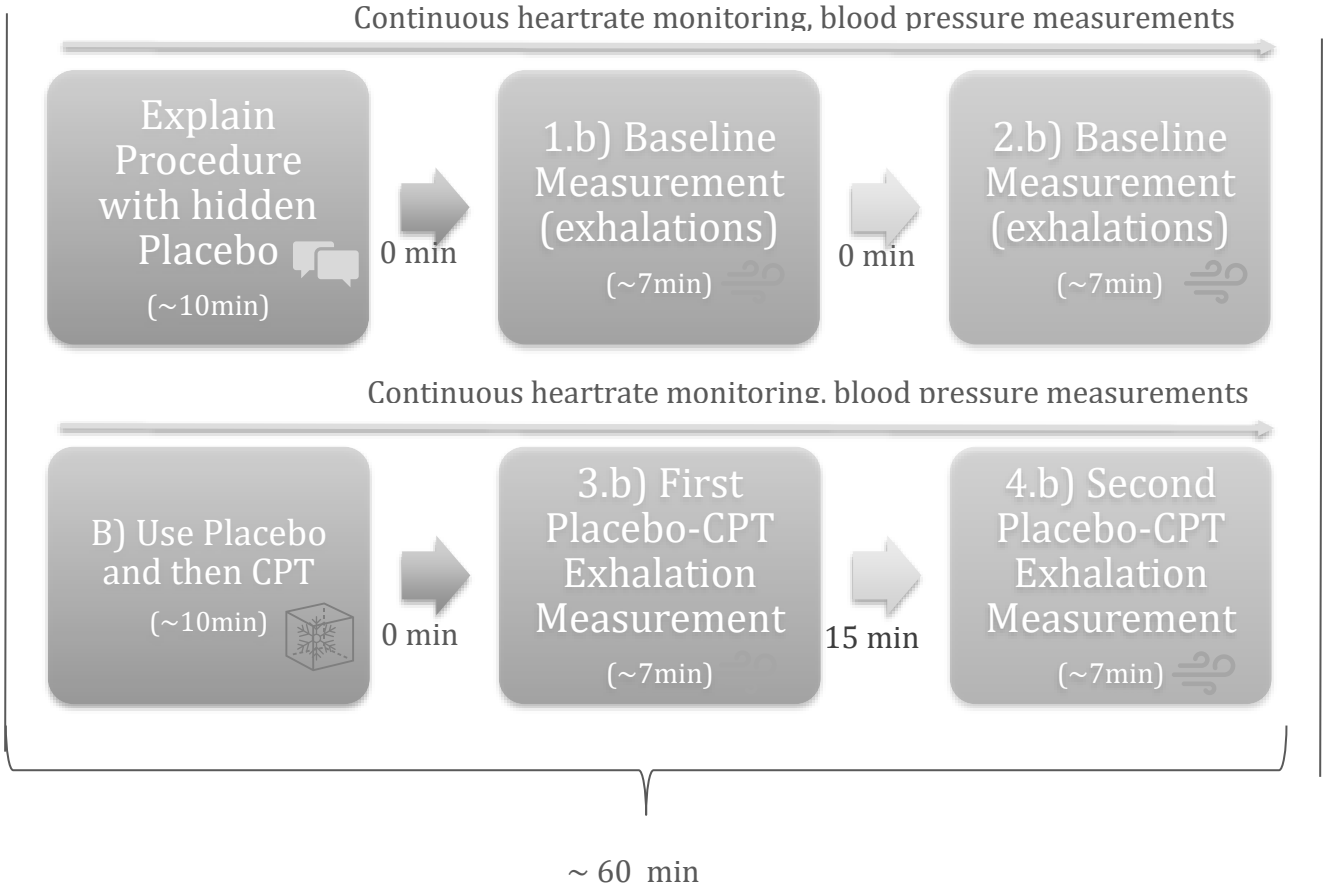

Figure 2. Schematic flowchart and process description of the study visit B

Cold Pressor Test (CPT)

The CPT is a widely used experimental technique for human pain induction, involving immersion of the hand or forearm in cold water. First documented as a test of cardiovascular reactivity (Hines & Brown, 1936), its application in investigation of pain perception, mechanisms, and treatment is due to a gradually mounting painful sensation of mild to moderate intensity. As water temperatures used are within the range considered noxious (below 15°C), nociceptors (pain receptors) are activated and transmit an aversive signal to the Central Nervous System (CNS). Like other pain inductions, the cold pressor allows fast and precisely controlled evaluations not possible in a clinical context (L.A., 2013). CPT is safely performed on thousands of participants (largest cohort up to date with 10'486 adult participants (Johansen, 2014)) including children every year worldwide and is generally well tolerated (Birnie & Noel, 2010). The principle is simple and safe, it is even employed in online self-administration research where 1876 participants self-administered CPT from their home (McIntyre MH, 2020).

Apparatus for the task is a tank of water of temperature of 2 °C (+-1 °C), with instruction to immerse the hand until too uncomfortable to continue. A maximum time limit per immersion of 3–5 min is applied. Quantitative measurement can then be made of pain threshold (point first perceived as painful) and tolerance time.

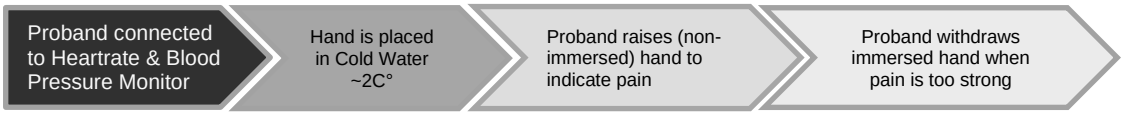

Figure 3. CPT Guide

3 STUDY POPULATION AND STUDY PROCEDURES

4.1 Inclusion and exclusion criteria, justification of study population

We will recruit 20 healthy subjects and comprise two study visits with at least 24 hours between the two visits. Participants will be subjected to minimally distressing tests and intervention procedures. Participants will be included in this study according to the following criteria:

Inclusion criteria:

- Healthy adult volunteers
- German speaking, or good knowledge of the German language
- Able to understand the study
- Able to give informed consent

Exclusion criteria:

- Regularly taking medication potentially interfering with pain sensation (analgesics, antihistamines and calcium and potassium channel blockers)
- Current pregnancy
- Daily smokers (as per WHO definition: A daily smoker is someone who smokes any tobacco product at least once a day.)
- Neuropathy
- Chronic pain (> 3 Months)

- Neuromuscular or psychiatric disease
- Known or suspected heart, kidney or liver disease
- Hypertension (Systolic (mmHg) >130, Diastolic (mmHg) >80)
- History of fainting or seizures
- History of Frostbite
- Current medications (psychoactive medication, narcotics, intake of analgesics) or being currently in psychological or psychiatric treatment.

4.2 Recruitment, screening and informed consent procedure

Participants will be recruited from the University campus/Hospital of Basel by word of mouth, flyers and via advertisement on the homepage of the University of Basel and social media. A minimum of 24 hours between recruitment procedure and decision of participant to participation is given. Due to the study design, the participants cannot be informed about all aspects of the procedure before the start of the study. Therefore, at the first Visit (randomly A or B), the investigators will explain to each participant the nature of the study as described in IC 1 (see appendix 2). Further, the procedures involved will be explained, the expected duration, the potential risks and benefits and any discomfort they may entail. The participants then sign IC 1. IC 1 will be signed and dated by the investigator or his designee at the same time as the participant sign. No medical records will be screened for this study.

Participants will receive CHF 100 for their participation in both study visits. After maximum 6 months or in case of withdrawal, the participants will be debriefed about the true aim of the study and sign IC 2 (see appendix 2) which they will receive via post. A copy of the signed informed consents will be given to the study participant. The consent forms will be retained as part of the study records.

4.3 Study procedures

The overall study duration will be: Recruitment start date: 01.10.2021, Study completion: 28.02.2023. The study comprises two visits (A and B) of 60 minutes each at the Breath Analysis laboratory of Prof. Sinues (UKBB). The breath analysis platform consists of a commercially available ion source (FIT, Spain) coupled to a high-resolution mass spectrometer (Thermo Scientific, Bremen). The process is completely non-invasive as the participant just exhales through a commercially available disposable bacterial/viral filter coupled to the ion source.

4.4 Withdrawal and discontinuation

We foresee the following reason for participants not to complete the study:

- Technical failure of the SESI-MS Ionization analytical platform
- Withdrawal of informed consent

In the case of withdrawal, the mass spectra and any material of the participant will be destroyed or deleted. In the case of drop-outs, we will replace the participants.

4 STATISTICS AND METHODOLOGY

5.1. Statistical analysis plan and sample size calculation

This is a pilot study and no literature has been found on the topic of placebo and metabolomics that may allow for a reasonable sample size estimation. Thus, we employ a rule of thumb approach based on our experiences in previous metabolomics studies (Pablo Sinues, 2013). (Pablo Sinues Tarokh L, 2014)

All 20 participants will undergo two study visits. The order in which they will receive differs, with participants being randomly split into two groups via matlab randi function: 10 participants first CPT + placebo and then CPT, and 10 participants vice versa. Null and alternative hypotheses are formulated as follows:

- Null hypothesis: the mean difference concentration (i.e. signal intensity as proxy) of exhaled metabolites is equal in CPT and in CPT+placebo ($\mu_{CPT} = \mu_{CPT+Placebo}$)
- Alternative hypothesis: the mean difference concentration (i.e. signal intensity as proxy) of exhaled metabolites is significantly different in CPT and in CPT+ placebo ($\mu_{CPT} \neq \mu_{CPT+Placebo}$)

A two-way repeated measures ANOVA will be performed to evaluate the effect of different treatments (CPT and CPT + placebo) over time on exhaled metabolite abundance. False discovery rate for multiple testing will be taken care using established procedures (Storey, 2002). The preprocessing of the raw mass spectra and the statistical analysis will be implemented using MATLAB (MathWorks, Massachusetts, US).

5.2. Handling of missing data and drop-outs

This is a pilot study looking at physiological outcomes, thus it will be analyzed on a per-protocol basis. In the case of drop-outs, we will simply replace the participants.

5 REGULATORY ASPECTS AND SAFETY

6.1 Local regulations / Declaration of Helsinki

This study is conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP the HRA as well as other locally relevant legal and regulatory requirements.

6.2 (Serious) Adverse Events and notification of safety and protective measures

An Adverse Event (AE) is any untoward medical occurrence in a patient or a clinical investigation subject which does not necessarily have a causal relationship with the trial procedure. An AE can therefore be any unfavourable or unintended finding, symptom, or disease temporally associated with a trial procedure, whether or not related to it.

A Serious Adverse Event (SAE) (ClinO, Art. 63) is any untoward medical occurrence that

- Results in death or is life-threatening,
- Requires in-patient hospitalisation or prolongation of existing hospitalisation,
- Results in persistent or significant disability or incapacity, or
- Causes a congenital anomaly or birth defect

Both Investigator and Sponsor-Investigator make a causality assessment of the event to the trial intervention, (see table below based on the terms given in ICH E2A guidelines). Any event assessed as possibly, probably or definitely related is classified as related to the trial intervention.

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

Both Investigator and Sponsor-Investigator make a severity assessment of the event as mild, moderate or severe. Mild means the complication is tolerable, moderate means it interferes with daily activities and severe means it renders daily activities impossible.

Reporting of SAEs (see ClinO, Art. 63)

All SAEs are documented and reported immediately (within a maximum of 24 hours) to the Sponsor-Investigator of the study.

If it cannot be excluded that the SAE occurring in Switzerland is attributable to the intervention under investigation, the Investigator reports it to the Ethics Committee via BASEC within 15 days.

Follow up of (Serious) Adverse Events

Notification of safety and protective measures (see ClinO, Art 62, b)

If immediate safety and protective measures have to be taken during the conduct of the study, the investigator notifies the Ethics committee of these measures, and of the circumstances necessitating them, within 7 days.

6.3 (Periodic) safety reporting

An annual safety report (ASR/DSUR) is submitted once a year to the local Ethics Committee by the Investigator (ClinO, Art. 43 Abs).

6.4 Radiation

NA

6.5 Pregnancy

NA

6.6 Amendments

Substantial changes to the study setup and study organization, the protocol and relevant study documents are submitted to the Ethics Committee for approval before implementation. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Ethics Committee. Such deviations shall be documented and reported to the Ethics Committee as soon as possible.

A list of all non-substantial amendments will be submitted once a year to the competent EC together with the ASR.

6.7 (Premature) termination of study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances, e.g.

- Ethical concerns,
- Insufficient participant recruitment,
- When the safety of the participants is doubtful or at risk (e.g. when the benefit-risk assessment is no longer positive),
- Alterations in accepted clinical practice that make the continuation of the study unwise, or
- Early evidence of harm or benefit of the experimental intervention

Upon regular study termination, the Ethics Committee is notified via BASEC within 90 days (ClinO, Art. 38).

Upon premature study termination or study interruption, the Ethics Committee is notified via BASEC within 15 days (ClinO, Art. 38).

6.8 Insurance

In the event of study-related damage or injuries, the liability of the institution provides compensation, except for claims that arise from misconduct or gross negligence.

7 FURTHER ASPECTS

7.1 Overall ethical considerations

This project entails two main ethical considerations: The participants will be exposed to a hidden placebo and the discomfort the CPT will cause on the participants.

7.2 Risk-benefit assessment

The risks in taking part in this trial are minimal. The breath analysis is non-invasive as the participants simply exhale into a disposable mouthpiece. The CPT can be stopped by the probands as soon as they feel discomfort. Potential light-headedness by probands is under control by a medical doctor on site and a chair is provided if the probands need to rest.

There is no direct benefit to the project participants, however, studying the biological mechanisms of the placebo effects have a great and overarching impact into the broad field of clinical interventions. Amongst others, placebo effects tend to obscure efficacy of drugs. Any new tool to reduce this unwanted data variability, would benefit clinical trials as well as clinical practice and patient care. Furthermore, it may also reduce patient's exposure to drugs they might not need, henceforward reducing drug side-effects in overall population.

Defining who might respond to placebo and how this happens biologically is of great importance as those who are very likely to respond to placebo could be excluded from clinical trials to increase the chances for seeing a drug-specific effect.

8 QUALITY CONTROL AND DATA PROTECTION

8.1 Quality measures

For quality assurance the sponsor, the Ethics Committee or an independent trial monitor may visit the research sites. Direct access to the source data and all study related files is granted on such occasions. All involved parties keep the participant data strictly confidential.

8.2 Data recording and source data

A designated research fellow will be responsible for data collection and data management. Data are registered in a paper Case Report Form (CRF). Paper documents and informed consent forms will be stored in a lock-secured cupboard in a dedicated research office in the responsible laboratory (UKBB, Basel). Only study investigators have access to these documents. Authorized staff of the responsible ethics committee can request access to these data for monitoring and auditing purposes.

Scientific reports generated from the study will not contain information that would identify the participating persons. After termination of the study records and documents pertaining to the conduct of this study, including CRFs, consent forms, laboratory test results and clinical notes will be archived for ten years and then destroyed. The UKBB Division of Pediatric Pulmonology will handle administration of the study database. The data entry will be performed by the study team. Authorized staff of the responsible ethics committee can request access to these data for monitoring and auditing purposes. Electronic data will be stored on password-secured computers. Files will be stored within the UKBB intranet server. The data is securely stored on the hospital server in form of mass spectra, exhalion data, heartrate monitor data and logfile excel lists

(Appendix 8). Data can only be accessed by authorized users from registered computers at the UKBB. Source data will consist of coded mass spectra.

8.3 Confidentiality and coding

Trial and participant data will be handled with uttermost discretion and is only accessible to authorised personnel who require the data to fulfil their duties within the scope of the study. On the CRFs and other study specific documents, participants are only identified by a unique participant number.

All personal information obtained for this study is confidential and disclosure to third parties other than those noted below is prohibited. Confidentiality of the subjects will be maintained by assigning subjects a study number (ID), keeping identifiers separate from the data and storing data in a locked file and secure computer database in line with Swiss legal requirements. Patient identifier will be assigned with a consecutive number after the project acronym (e.g. CPT_001). All recorded electronic data (i.e. mass spectra, blood pressure, heartrate) will be coded with the patient identifier. These source data will be recorded in password-protected local computers and back-up in an external hard drive.

8.4 Retention and destruction of study data and biological material

No storage of biological material is foreseen. Records and documents pertaining the conduct of this study, including CRFs, consent forms, participant diaries and clinical notes will be retained for 10 years.

9 MONITORING AND REGISTRATION

The aim of monitoring is to evaluate the progress of the study, to verify the accuracy and completeness of CRFs, to ensure that all protocol requirements, applicable local authority regulations and investigator's obligations are being fulfilled, and to resolve any inconsistencies in the study records. Regular monitoring visits at the investigator's site prior to the start and during the course of the study will be performed by independent monitors not included in the research group. The monitoring is performed by Isabel Gonzales (experienced and GCP certified lead study nurse UKBB, USB). The source data/documents are accessible to monitors and questions are answered during monitoring

The study is registered with BASEC, the FOPH portal SNCTP (Swiss National Clinical Trial Portal) and clinicaltrials.gov.

10. FUNDING / PUBLICATION / DECLARATION OF INTEREST

This project will be supported by the Fondation Botnar via the Professorship of the principal investigator and the Swiss National Science Foundation (173168). The compensation of participants will be covered by the Division of Clinical Psychology and Psychotherapy, Faculty of Psychology, University of Basel, which also supports the study with PhD candidates and master students.

We plan to publish the results of this study in a peer reviewed journal. We also plan to present the results of this study at clinical conferences.
Authorship credit is based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.
Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.
We declare no conflict of interest (independence, intellectual, financial, proprietary).

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Appendix 1: Schedule of assessments

Visit	1	2
Time	60 min	60 min
Oral information	+	+
IC 1	+	
IC 2		+
Check inclusion-/ exclusion criteria	+	
CPT	+	+
Self-Report Pain	+	+
Heart rate Monitoring	+	+
Blood Pressure Monitoring	+	+
Breath analysis	+	+
Placebo		+

(uploaded to BASEC system):
Appendix 2: IC 1&2
Appendix 3: Information zur Prüfsubstanz
Appendix 4: Participant recruitment Flyer
Appendix 5: CRF
Appendix 6: GC
Appendix 7: script 'medication'
Appendix 8: logfile data
Appendix 9: inclusion/exclusion checklist