Short Term Effect of Glucocorticoids on Brown Adipose Tissue Thermogenesis in Humans – a randomized placebo-controlled interventional study

Clinical Study Protocol

Short Title: GlucoBAT Study

Study Type:	Clinical trial with Investigational Medicinal Product (IMP)
Study Categorisation:	B
Study Registration:	Anticipated in clinicaltrials.gov and KOFAM
Study Identifier:	EKNZ 2016-01859
Sponsor, Sponsor-Investigator or Principal Investigator:	PD Dr. Matthias Betz Klinik Endokrinologie, Diabetologie und Metabolismus Universitätsspital Basel Petersgraben 4 4031 Basel phone: 0041 61 5565654 fax: 0041 61 2655100 email: Matthias.Betz@usb.ch
Investigational Product:	Prednisone 20 mg Galepharm
Protocol Version and Date:	2.1, 2018-07-31

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Signature Page(s)

Study number	***NCT03269747***
Study Title	Short Term Effect of a Glucocorticoids on Brown Adipose Tissue Thermogenesis in Humans – a randomized placebo-controlled interventional study

The Sponsor-Investigator has approved the protocol version 2.1, dated 2018-07-31, and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Sponsor-Investigator:

PD Dr. Matthias Betz

Basel, 31.07.2018

Place/Date	Signature

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STUDY SYNOPSIS

Sponsor / Sponsor-Investigator	University Hospital Basel, Department of Endocrinology, Diabetes and Metabolism PD Dr. Matthias Betz
Study Title:	Short Term Effect of Glucocorticoids on Brown Adipose Tissue Thermogenesis in Humans
Short Title / Study ID:	GlucoBAT study
Protocol Version and Date:	2.1, 2018-07-31
Trial registration:	Anticipated study registry: clinicaltrials.gov, KOFAM
Study category and Rationale	Category B, prednisone is approved by Swissmedic but will not be used according to prescribing information, placebo will be used in a cross-over design
Clinical Phase:	Phase 4 (according to ICH E8 para 3.1.3)
Background and Rationale:	Active brown adipose tissue (BAT) has recently been unambiguously discovered in human adults. Active BAT increases energy expenditure and improves glucose tolerance. Pharmacological use of glucocorticoids (GCs) is widespread in clinical practice due to their high anti-inflammatory efficacy. While short-term administration even of high doses usually is well tolerated, long-term use of medium to high amounts of GCs leads to unfavorable metabolic changes, characterized by an increase in intra-abdominal fat mass, a decrease in muscle mass and insulin resistance. In line with these well-known side-effects of GCs, several in vitro studies and animal models demonstrate an inhibiting effect of GCs on BAT thermogenesis.
Objective(s):	The aim of this study is to determine the short term effects of prednisone on BAT thermogenesis in humans.
Outcome(s):	Primary outcome: - Cold induced thermogenesis: Increase in energy expenditure above resting metabolic rate in response to a mild cold stimulus. Secondary outcomes: - MRI determined fat fraction of supraclavicular BAT - MRI determined volume of supraclavicular BAT - Supraclavicular skin temperature in response to mild cold stimulus - cold stimulated FGD uptake in brown adipose tissue, determined as SUV_{mean} by FDG-PET/CT - SUV_{max} in the supraclavicular adipose tissue depot Other outcomes: - Glucose and insulin level before and after mild cold stimulus - FGF21 level before and after mild cold stimulus - Finger tip temperature in response to mild cold stimulus as a measure of peripheral vasoconstriction. - LDL and triglyceride levels after each treatment period - Expression levels of genes involved in thermogenesis and white to brown adipose tissue transdifferentiation in supraclavicular adipose tissue. - Expression levels of genes involved in thermogenesis and calcium cycling in vastus lateralis muscle.

Study design:	Randomized, double-blind crossover study
Inclusion / Exclusion criteria:	Inclusion criteria - Healthy male volunteers - BMI between 19-27 kg/m2 - Age between 18 and 40 years Exclusion criteria: - Cold induced thermogenesis of less than 5% basal metabolic rate (determined during screening visit) - Contraindications to the class of drugs under study, e.g. known hypersensitivity or allergy to class of drugs or the investigational product, - History of depressive disorder, anxiety disorder - History of tuberculosis or latent infection - Increased intraocular pressure - History of peptic / gastrointestinal ulcer disease - Concomitant medication: Non-steroidal anti-inflammatory drugs (NSAID), other glucocorticoids, diuretics, antihypertensives, fibrates or statins, metformin - Other clinically significant concomitant disease states (e.g., renal failure, hepatic dysfunction, cardiovascular disease, diabetes mellitus), - Hypersensitivity to cold (e.g. Raynaud Syndrome) - Allergy to local anesthetic - Known or suspected non-compliance, drug or alcohol abuse - Inability to follow the procedures of the study - Participation in another study with investigational drug within the 30 days preceding and during the present study, - Previous enrolment into the current study, - Enrolment of the investigator, his/her family members, employees and other dependent persons - Hypothyroidism without sufficient substitution - Claustrophobia - MRI incompatible implants - Enrolment into another study using ionizing radiation within the previous 12 months. Reason for inclusion of healthy volunteers In order to limit the sample size and perform this proof-of-principle study we need to limit the heterogeneity of the study population. Therefore it is necessary to perform the study in healthy volunteers that have a substantial baseline amount of cold-induced thermogenesis.

Measurements and procedures:	The study will be carried out as a randomized, double-blinded cross-over trial in healthy volunteers. During both the placebo and verum phase we will assess BAT function using indirect calorimetry, MRI, skin temperature measurement and we will perform ^{18}F-FDG-PET/CT of supraclavicular BAT. The potential of the tissue to produce heat upon a mild cold stimulus will be assessed non-invasively by using indirect calorimetry during rest at warm temperatures and after a mild cold stimulus of 120 min duration. During cold exposure, subjects will receive an injection of ^{18}F-FDG and glucose uptake into BAT will be measured by PET-CT. Concomitantly, plasma will be sampled and analysed for metabolic parameters such as glucose and plasma lipids. Specifically the following study visits are planned (to be performed both during verum and pacebo phase): - after 7 d of exposure to study drug or placebo, respectively: cold induced thermogenesis, and skin temperature in response to mild cold exposure - MRI of supraclavicular BAT and needle biopsies of supraclavicular adipose tissue and vastus lateralis muscle - Measurement of glucose uptake into BAT by FGD-PET/CT
Study Product / Intervention:	Prednisone Galepharm 20 mg, 2 tablets once daily for 7 days
Control Intervention (if applicable):	corresponding placebo for 7 days
Number of Participants with Rationale:	16 Based on previous results, we assume a mean CIT of 150 kcal/d (approximately 10% of REE during warm conditions), a standard deviation of 0.5 times mean and a correlation between groups (prednisone vs. placebo) of 0.65. In order to be able to detect a difference in CIT of -33%, which we consider clinically significant, we will need 13 participants to obtain a statistical power of > 80% (analysis by Wilcoxon signed-rank test, not assuming normal distribution of data, $\alpha<0.05$). To account for drop-outs and sample heterogeneity we plan to include 16 participants.
Study Duration:	12-18 months
Study Schedule:	10/2017 First-Participant-In (planned) 04/2020 Last-Participant-Out (planned)
Investigator(s):	PD Dr. Matthias J. Betz University Hospital of Basel Department of Endocrinology, Diabetes and Metabolism Petersgraben 4, 4031 Basel, Switzerland Tel. 0041 61 265 5078 Fax. 0041 61 265 5100 Email: Matthias.Betz@usb.ch
Study Centre(s):	Single centre University Hospital of Basel, Department of Endocrinology, Diabetes and Metabolism. 4031 Basel. Switzerland
Statistical Considerations:	The primary endpoint of the study will be analysed using a Wilcoxon signed rank test. For sample size estimation see above.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP or ISO EN 14155 (as far as applicable) as well as all national legal and regulatory requirements.

ABBREVIATIONS

AE	Adverse Event
BAT	Brown adipose tissue
CA	Competent Authority (e.g. Swissmedic)
CEC	Competent Ethics Committee
CIT	cold induced thermogenesis
ClinO	Ordinance on Clinical Trials in Human Research *(in German: KlinV)*
CRF	Case Report Form
CTCAE	Common terminology criteria for adverse events
DSUR	Development safety update report
eCRF	Electronic Case Report Form
GC	Glucocorticoid
GCP	Good Clinical Practice
H1	Alternative hypothesis
HFG	Humanforschungsgesetz (Law on human research)
HMG	Heilmittelgesetz
Ho	Null hypothesis
HRA	Federal Act on Research involving Human Beings
IB	Investigator's Brochure
IIT	Investigator-initiated Trial
IMP	Investigational Medicinal Product
ISO	International Organisation for Standardisation
ITT	Intention to treat
KlinV	Verordnung über klinische Versuche in der Humanforschung
LLN	Lower limit of normal
LPTh	Loi sur les produits thérapeutiques
LRH	Loi fédérale relative à la recherche sur l'être humain
MD	Medical Device
MRI	Magnetic resonance imaging
PET-CT	positron emission tomography – computed tomography
PI	Principal Investigator
REE	resting energy expenditure
SDV	Source Data Verification
SOP	Standard Operating Procedure
SPC	Summary of product characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File

TP	Treatment period
ULN	Upper limit of normal

STUDY SCHEDULE

See 9.1, page 24

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor, Sponsor-Investigator

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1.4 Laboratory

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1.5 Monitoring institution

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Universitätsspital Basel, Spitalstrasse 12, CH-4031 Basel

1.6 Data Safety Monitoring Committee

Clinical Trial Unit (CTU) Basel
Universitätsspital Basel, Spitalstrasse 12, CH-4031 Basel

1.7 Any other relevant Committee, Person, Organisation, Institution

Calculation of radiation exposure
Lisa McDougall, Radiopharmacy, Dept. of Radiology and Nuclearmedicine, University Hospital Basel.

2. ETHICAL AND REGULATORY ASPECTS

Before the study will be conducted, the protocol, the proposed patient information and consent form as well as other study-specific documents shall be submitted to a properly constituted Competent Ethics Committee (CEC) and competent authority (Swissmedic) in agreement with local legal requirements, for formal approval. Any amendment to the protocol must as well be approved (if legally required) by these institutions.

The decision of the CEC and Swissmedic/foreign competent authority concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

2.1 Study registration

The study will be registrated in clinicaltrials.gov and in the Swiss Federal Complementary Database (KOFAM).

2.2 Categorisation of study

Category B:

Prednisone is approved in Switzerland for the treatment of inflammatory and autoimmune diseases. However, it will be used in healthy volunteers and compared to placebo.

2.3 Competent Ethics Committee (CEC)

The responsible investigator at each site ensures that approval from an appropriately constituted Competent Ethics Committee (CEC) is sought for the clinical study.

All changes in the research activity and all unanticipated problems involving risks to humans (including in case of planned or premature study end and the final report) are reported within the allowed time frame to the CEC and no changes are made to the protocol without prior Sponsor and CEC approval, except where necessary to eliminate apparent immediate hazards to study participants.

Premature study end or interruption of the study is reported within 15 days. The regular end of the study is reported to the CEC within 90 days, the final study report is submitted within one year after study end. Amendments are reported according to chapter 2.10.

2.4 Competent Authorities (CA)

The Sponsor will obtain approval from the competent authority (Swissmedic) before the start of the clinical trial.

Premature study end or interruption of the study is reported within 15 days to CA. The regular end of the study is reported to the CA within 90 days, the final study report is submitted within one year after study end. Amendments are reported according to chapter 2.10. Non-substantial amendments are reported as soon as possible.

2.5 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, the Swiss Law and Swiss regulatory authority's requirements. The CEC and regulatory authorities will receive annual safety and interim reports and be informed about study stop/end in agreement with local requirements.

2.6 Declaration of interest

PD Dr. Matthias Betz declares no conflict of interest.

2.7 Patient Information and Informed Consent

The investigators (or his designee) will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment.

The participant must be informed that his/her medical records may be examined by authorised individuals other than their treating physician.

All participants for the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an informed decision about their participation in the study. Enough time (at least 24 hours) will be given to the participant to decide whether to participate or not.

The patient information sheet and the consent form will be submitted to the CEC and to the competent authority (as applicable) to be reviewed and approved. The formal consent of a participant, using the approved consent form, must be obtained before the participant is submitted to any study procedure.

The participant should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

2.8 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject

identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Sponsor (-Investigator), a competent authority (e.g. Swissmedic), or an ethics committee may require direct access to parts of the medical records relevant to the study, including participants' medical history.

2.9 Early termination of the study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention

2.10 Protocol amendments

The Sponsor-Investigator and the Principal Investigator are allowed to amend the protocol or to provide suggestions for a protocol amendment.
Substantial amendments are only implemented after approval of the CEC and CA respectively.
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 sponsor and the CEC/CA. Such deviations are documented and reported to the sponsor and the CEC/CA as soon as possible.

All Non-substantial amendments are communicated to the CA as soon as possible and to the CEC within the Annual Safety Report (ASR).

3. BACKGROUND AND RATIONALE

3.1 Background and Rationale

Brown adipose tissue (BAT) is a thermogenic tissue that can convert chemical energy stored in triglycerides directly into heat due to the unique protein UCP1 (uncoupling protein). It is located in the inner mitochondrial membrane of brown adipocytes and can upon activation short-circuit the respiratory chain leading to uncoupling of cellular respiration from ATP production and an increase in energy expenditure[1]. While its importance for small mammals and human infants had been known for decades, it was only in 2009 that the presence and metabolic activity of BAT was unambiguously discovered in human adults [2-4]. Both in basic animal studies and in human trials BAT activity leads to an increase in energy expenditure and importantly to increased insulin sensitivity, as the tissue takes up glucose from the circulation. Importantly, the BAT depots in human adults consist of so called "beige" adipocytes that can arise in white adipose tissue (WAT) depots upon regular cold exposure. These cells have are of a different lineage when compared to classical brown adipocytes but share the morphologic and functional properties of the latter[5]. In animals, induction of beige adipocytes in WAT depots has been shown to ameliorate obesity and improve insulin sensitivity.

Pharmacological use of glucocorticoids (GCs) is widespread in clinical practice due to their high anti-inflammatory efficacy. While short-term administration even of high doses usually is well tolerated, long-term use of medium to high amounts of GCs leads to unfavorable metabolic changes, characterized by an increase in intra-abdominal fat mass, a decrease in muscle mass and insulin resistance [6, 7].

3.2 Investigational Product and Indication

Prednisone is approved for use in Switzerland. The main indications are allergic, autoimmune and inflammatory diseases. The recommended starting dose for acute, non-life threatening diseases is 15-30 mg/d [8].

3.3 Preclinical Evidence

In line with these well-known side-effects of GCs, several in vitro studies and animal models

demonstrate an inhibiting effect of GCs on BAT thermogenesis. While certain amounts of GCs are necessary for the differentiation of brown adipocytes [9] and UCP1 expression [10], even short-term (96 h) exposure of rats to high doses of corticosterone significantly reduced UCP1 expression in BAT and blunted the norepinephrine induced increase in UCP1 expression as a measure of BAT capacity [11, 12]. In vitro glucocorticoids potently inhibited the expression of UCP1 in a brown adipocyte cell line and this effect could be antagonized by the glucocorticoid-receptor antagonist RU486 [13], which has recently also been demonstrated in mice in vivo [14]. In mice, dexamethasone treatment not only led to increased obesity and hepatic steatosis but also decreased BAT UCP1 content and β3-adrenoreceptor-agonist mediated increase in oxygen consumption, which is a measure of BAT thermogenic capacity [15].

Additionally, local availability of glucocorticoids is regulated by the 11-β-hydroxysteroid dehydrogenase (HSD) shuttling-system; 11-β-HSD type 1 is expressed in white adipose tissue (WAT) and increases local availability of cortisol by conversion of cortisone. Overexpression of 11-β-HSD1 in brown adipocytes decreased markers of BAT differentiation while administration of an antagonist led to the opposite effect both in vitro and in vivo [16]. In a rat model of diet induced obesity an 11-β-HSD1 antagonist led to improved triglyceride uptake and oxidative metabolism in BAT as well as to a substantial increase of UCP1 expression [17]. More importantly, a recently published study demonstrated direct effects of dexamethasone on the development of brown adipocytes within WAT depots (so called “beige” BAT). Dexamethasone binding to the glucocorticoid receptor (GR) transcriptionally activates the microRNA miR-27b, which inhibits differentiation of “beige” adipocytes and is repressed upon cold exposure in mice under physiological conditions [18, 19]. The inhibitory effect of GCs on the development of “beige” BAT might be especially relevant, as the major BAT depots in human adults are of this tissue type [20, 21]. Recently, novel uncoupling mechanisms independent of UCP1 have been described: In beige adipocytes creatine / phospho-creatine-cycling contributes to energy expenditure and can compensate loss of UCP1 in mice [22]. These findings are in line with studies in human beige adipose tissue samples indicating higher expression of mitochondrial creatine kinases CKMT1[23].

Additionally, muscle may contribute significantly to non-shivering thermogenesis through the Sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) system, which can use rapid calcium cycling as futile cycling and is regulated by sarcolipin [24]. This is underscored by a recent paper demonstrating improved glucose tolerance in humans suffering from type 2 diabetes after repetitive exposure to mild cold and increased expression of GLUT4 in skeletal muscle associated with an increased cold induced glucose uptake into muscle [25]. Interestingly, there is currently only one paper on the effect of glucocorticoids on SERCA and sarcolipin in muscle describing reduced expression of SERCA 1 and 2 in rats [26].

3.4 Clinical Evidence to Date

Previous prospective studies investigating the short-term effect of glucocorticoids in humans revealed no significant differences in resting energy expenditure [27-29]. Recently, a small study of acute exposure to glucocorticoid over 24 hours demonstrated increased BAT activity in human volunteers[30], which is contrary to our hypothesis. Glucocorticoids are well known to increase acutely adrenergic response in human lung both due to acutely increased transcription of adrenergic receptors[31] and increased activity of adenylate cyclase[32]. Importantly, both effects seem to be transient with a maximum after 24 hours.

In clinical practice GCs are often administered over periods ranging from one week to several months, making the investigation of the short term (e.g. one week) and long term (several months) effects practically relevant. We hypothesize, however, that in short term (e.g. 7 days) and long-term treatment the inhibiting effects outlined above might dominate.

3.5 Dose Rationale

The recommended starting dose for prednisone in acute, non-life threatening diseases is 15-30 mg/d. We aim for a dose of 40 mg per day as this is a common dose used in clinical practice (e.g. in acute exacerbations of chronic obstructive lung disease) and is still well tolerated.

3.6 Explanation for choice of placebo

Prednisone will be tested against placebo in a randomized cross-over design. As the study participants

will be healthy volunteers, i.e. not suffer from inflammatory or autoimmune disease, placebo is ethically justified.

3.7 Risks / Benefits

Prednisone is generally well tolerated if taken short-term, i.e. shorter than two weeks, and the risk for adverse drug reactions is low[8].

However, the following adverse drug effects (among others) may occur especially after long term exposure:

Leucocytosis

Thromboembolism

Activation of latent infection, masking of inflammatory reactions

Cushing syndrome

Muscle wasting

Osteoporosis

Sodium retention, edema and hypertension

We will therefore limit the exposure to the study drug to one week.

In order to mitigate the potential risks we will ensure that participants are healthy before starting the investigational product. Volunteers will be fully informed about the potential side effects and should refrain from strenuous muscular exercise during the study period. Specifically volunteers will be excluded from participation if they have a history of the following diseases / conditions:

- Arterial hypertension
- Tuberculosis or other latent infection
- Depression or other mental disorders (psychosis, anxiety disorder)
- Peptic ulcer disease
- Diabetes mellitus or impaired fasting glucose
- Osteoporosis
- Kidney disease
- Liver disease
- Glaucoma (increased intra-ocular pressure)

Furthermore, participants in the study must not take the following medications during the course of the study

- Non-steroidal anti-inflammatory drugs (NSAID)
- Other glucocorticoids
- Diuretics
- Antihypertensives
- Fibrates or Statins
- Metformin

Risks associated with imaging procedures:

As the primary outcome will be quantified by ^{18}F-FDG-PET/CT participants will be exposed to ionizing radiation. According to Art. 2 ClinO the risk from ionizing radiation is deemed minimal if the total radiation dose is below 5 mSv for the research project.

Details on the radiation exposure associated with this study protocol are given under paragraph 10.1.4.

There will be no immediate benefit for the study participants. Participants will be therefore compensated for the time necessary to participate in the study.

As glucocorticoids are among the most widely used drugs worldwide, it is important to elucidate the effects of these substances on brown adipose tissue.

Risks associated with biopsy procedures:

Biopsies will be taken using a 2.5 mm core biopsy needle. Due to the small diameter of the needle trauma to tissue and associated risks will be minimal. However, the following risk need to be considered:

- Allergic reaction to local anaesthetic
- Bleeding at puncture site
- Infection
- Lesion of a cutaneous nerve

In order to mitigate the risks, care will be taken to use sterile procedures apply due pressure to the puncture site after the procedure.

3.8 Justification of choice of study population

In order to investigate the effects of prednisone on human BAT and minimize the number of subjects enrolled into the study as well as the exposure to the study drug and ionizing radiation we will need a study population without confounding comorbidity and/or medication thereby minimizing variability. We therefore plan to perform the study in healthy, male volunteers.

4. STUDY OBJECTIVES

4.1 Overall Objective

The overall objective of this study is to evaluate whether prednisone influences BAT activity in humans.

4.2 Primary Objective

The study seeks primarily to determine the effect of prednisone on BAT activity upon mild cold exposure as compared to placebo.

4.3 Secondary Objectives

The secondary objectives are to determine the effect of prednisone on cold induced thermogenesis and resting metabolic rate.

4.4 Safety Objectives

This does not apply as prednisone is already approved as a safe and effective anti-inflammatory drug.

5. STUDY OUTCOMES

5.1 Primary Outcome

Cold induced thermogenesis: Increase in energy expenditure above resting metabolic rate in response to a mild cold stimulus.

Relevance: this is the functional consequence of BAT activity leading to increased energy expenditure in response to mild cold

5.2 Secondary Outcomes

- Fat content (fat fraction) of supraclavicular BAT as determined by MRI
  Relevance: The fat-fraction of supraclavicular BAT has been shown to correlate well to the thermogenic capacity in human adults[33]
- Volume of supraclavicular BAT as determined by MRI
  Relevance: glucocorticoid treatment might also influence the size of BAT depots

- Cold induced 18F-FDG uptake (SUVmean) into the supra-clavicular brown adipose tissue as determined by 18F-FDG PET-CT after treatment with prednisone vs. placebo.
- SUV_{max} in the supraclavicular adipose tissue depot
  Relevance: 18F-FDG PET-CT is currently the gold-standard to evaluate BAT-function. It is the most sensitive measure of BAT activity.
- Supraclavicular skin temperature in response to mild cold stimulus
  Relevance: This is a surrogate parameter of BAT activation, which is easily detectable and

can give information about the time course of BAT activation during the cold exposure

5.3 Other Outcomes of Interest

- Glucose and insulin level before and after mild cold stimulus
  Relevance: BAT has been shown to take up glucose in response to cold stimulation and the amount of BAT could influence the glycaemic response to glucocorticoids.
- FGF21 level before and after mild cold stimulus
  Relevance: previous studies have shown that BAT activity in humans is positively correlated to FGF21 plasma levels [34]
- Finger tip temperature in response to mild cold stimulus as a measure of peripheral vasoconstriction.
  Relevance: This is a non-invasive measurement of vasoconstriction in response to cold exposure
- LDL and triglyceride levels after each treatment period
  Relevance: BAT has been shown to take up considerable amounts of plasma lipids when stimulated.
- Expression levels of genes involved in thermogenesis and white to brown adipose tissue transdifferentiation in supraclavicular adipose tissue.
  Relevance: supraclavicular adipose tissue can transdifferentiate to brown / beige adipocytes upon cold exposure and constitutes the main brown adipose tissue in human adults. We want to investigate the effect of prednisone on key transcription factors involved in this process.
- Expression levels of genes involved in thermogenesis and calcium cycling in vastus lateralis muscle.
  Relevance: muscle tissue contributes to non-shivering thermogenesis, probably due to futile calcium cycling. We want to study the effect of prednisone on key genes involved in this process.

5.4 Safety Outcomes

Does not apply.

6. STUDY DESIGN

6.1 General study design and justification of design

The study will be designed as a prospective placebo-controlled cross-over study in 16 healthy male volunteers.

Participants will receive prednisone 40 mg or placebo, respectively, once daily for one week. This will be followed by a wash-out period of at least 4 weeks followed by another week of study drug or placebo, respectively.

Both investigators and participants will be blinded to the sequence of study drug and placebo.

Figure 1 (page 11) gives an overview of the study flow.

6.2 Methods of minimising bias

6.2.1 Randomisation

After successful screening, participants will be randomized to be given prednisone or placebo in random order. The order of the two treatments is randomly predefined in a randomization list (produced by the Spitalpharmazie Unispital Basel) and is not known to the participants, the investigators and the study nurses involved in the trial.

Participants' treatment with prednisone or placebo, respectively, will be separated by a wash-out period of at least 4 weeks.

6.2.2 Blinding procedures

Prednisone and placebo will be administered as identically looking tablets. The packaging of prednisone and placebo will be performed by the Hospital Pharmacy of the University Hospital Basel according to a computer based randomization list generated by the Hospital Pharmacy. Analysis of the study

endpoints will be performed with the investigators and data analysts blinded to whether the participant took prednisone or placebo prior to the respective study visit, i.e. all end-point relevant measurement data will be entered into the eCRF database before the randomization is revealed.

6.2.3 Other methods of minimising bias

Does not apply.

6.3 Unblinding Procedures (Code break)

In emergency situations, e.g. adverse events, and up to the decision of the investigator if medically important, it is allowed to break the blind of the participant and to reveal the code. This will be performed by an independent physician from the Department of Endocrinology (see section 6.2) who will have access to the randomisation list. For the purpose of emergency unblinding purposes, a sealed envelope with the randomization data will be stored in a locked place in the Department of Endocrinology and be accessible to an independent physician 24/7. In case of an adverse event potentially linked to the study medication, the investigator will inform the independent physician and ask him/her to un-blind the participant.

7. STUDY POPULATION

The study will be performed at the Department of Endocrinology, Diabetes and Metabolism and the Department of Nuclear Medicine of the University Hospital Basel.

7.1 Eligibility criteria

Participants fulfilling all of the following inclusion criteria are eligible for the study, for example:

- Male sex
- Age 18 to 40 years
- BMI 19 to 27 kg/m^2
- Informed Consent as documented by signature (Appendix Informed Consent Form)

The presence of any one of the following exclusion criteria will lead to exclusion of the participant:

- Cold induced thermogenesis of less than 5% basal metabolic rate (determined during screening visit)
- Contraindications to the class of drugs under study, e.g. known hypersensitivity or allergy to class of drugs or the investigational product,
- History of depressive disorder, anxiety disorder
- History of tuberculosis or latent infection
- Increased intraocular pressure
- History of peptic / gastrointestinal ulcer disease
- Concomitant medication: Non-steroidal anti-inflammatory drugs (NSAID), other glucocorticoids, diuretics, antihypertensives, fibrates or statins, metformin
- Other clinically significant concomitant disease states (e.g., renal failure, hepatic dysfunction, cardiovascular disease, diabetes mellitus),
- Hypersensitivity to cold (e.g. Raynaud syndrome)
- Allergy to local anesthetic
- Known or suspected non-compliance, drug or alcohol abuse,
- Inability to follow the procedures of the study, e.g. due to language problems, psychological disorders, dementia, etc. of the participant,
- Participation in another study with investigational drug within the 30 days preceding and during the present study,
- Previous enrolment into the current study,
- Enrolment of the investigator, his/her family members, employees and other dependent persons,
- Hypothyroidism without sufficient substitution
- Claustrophobia
- MRI incompatible implants

- Enrolment into another study using ionizing radiation within the previous 12 months.

Lab parameters

- Lymphopenia: Lymphocyte count below lower limit of normal (LLN)
- Serum-Creatinine: above 1.5x upper limit of normal (ULN)
- ASAT, ALAT: above 2x upper limit of normal (ULN)
- Serum-Potassium: below 3.4 mmol/l
- Glycated Hemoglobin (HbA1c): above 6.0%
- Cholesterol / Triglyceride panel: outside normal limits
-

7.2 Recruitment and screening

(ClinO, Art 25, Appendix 3, 1.4 & 1.6; AGEK 5.1; SPIRIT #15)

Participants will be recruited by means of advertisements (online at “Marktplatz Uni Basel”). Interested volunteers contact the study investigators by email or telephone and will subsequently receive the ‘’Probandeninformation’’ and the ‘’Einverständniserklärung’’ via mail or e-mail. Willing to participate, they contact the investigators and an appointment for the screening visit is arranged.

The screening visit will comprise a health related questionnaire and an assessment of cold stimulated thermogenesis (CIT). It will be measured as described below in order to determine whether the prospective participant has sufficient brown adipose tissue in order to participate in the study.

If participants fulfil the safety criteria and react to cold with a CIT of 5% or more of basal metabolic rate a laboratory analysis consisting of blood count, liver and kidney function, and thyroid function. If participants fulfil all exclusion/inclusion criteria they can participate in the study.

At the end of the study, study participants will receive a total of 900 CHF as a compensation for the time effort.).

7.3 Assignment to study groups

The hospital pharmacy of the University Hospital Basel will provide computer-generated randomization lists. Participants will be entered into an electronic study system which assigns the respective study number. Study drug or placebo will be supplied in numbered glass bottles produced by the hospital pharmacy of the University Hospital Basel.

7.4 Criteria for withdrawal / discontinuation of participants

Participants will be withdrawn from the study in the following situations:

- Participants withdraws his/her consent
- Non-compliance to study procedures
- Elevation of liver enzymes above 3x the ULN.
- Evidence of allergy to study drug / placebo.

Participants withdrawn from the study will be replaced.

8. STUDY INTERVENTION

8.1 Identity of Investigational Products (treatment)

8.1.1 Experimental Intervention (treatment)

Prednisone 20 mg, tablets.

8.1.2 Control Intervention (standard/routine/comparator treatment / medical device)

Identically looking placebo tablets

8.1.3 Packaging, Labelling and Supply (re-supply)

Study drug will be produced by Galepharm, Switzerland. Identically looking placebo tablets, “P-Tablette

Weiss, Liechtenstein" will be obtained from Winthrop Arzneimittel GmbH, 65927 Frankfurt am Main, Germany. Randomization and packaging for the study will be provided by the hospital pharmacy of the University Hospital Basel.

8.1.4 Storage Conditions

Verum and placebo tablets will be stored in a locked place at room temperature (15-25°C) protected from light.

8.2 Administration of experimental and control interventions

8.2.1 Experimental Intervention

Two tablets of prednisone 20 mg taken once daily by mouth in the morning, for seven consecutive days. Identically looking containers with study drug or placebo, respectively, will be handed out to participants on visit 2 or 5, respectively. Participants will be asked to take two tablets each morning. On days with study visits, tablets will be taken at the beginning of the study visit.

8.2.2 Control Intervention

Two identically looking placebo tablets once daily by mouth in the morning, for seven consecutive days.

Identically looking containers with study drug or placebo, respectively, will be handed out to participants on visit 2 or 5, respectively. Participants will be asked to take two tablets each morning. On days with study visits, tablets will be taken at the beginning of the study visit.

8.3 Dose modifications

Does not apply

8.4 Compliance with study intervention

Participants will receive a drug diary and be asked to bring the drug diary and study drug containers to each study visit.

8.5 Data Collection and Follow-up for withdrawn participants

Data will only be collected from participants who complete the study according to the protocol.

8.6 Trial specific preventive measures

Levels of glucose and glycated haemoglobin (A1c) will be measured at the screening visit in order to reduce the risk of hyperglycemia.

In order to minimize adverse reactions participants must specifically not take the following medications during the study period:

- Non-steroidal anti-inflammatory drugs (NSAID)
- Other glucocorticoids
- Diuretics
- antihypertensives

8.7 Concomitant Interventions (treatments)

Whenever possible, any additional treatment during study period should be avoided. If concomitant care is strongly recommended, the investigator decides whether the study can be continued.

If concomitant medication is taken, their use will be documented in the CRF.

8.8 Study Drug Accountability

From shipment to the site until return or disposal, study drugs are accurately and adequately monitored. Dates of receipt/expiry/use/return are recorded.

8.9 Return or Destruction of Study Drug

At the end of the study remaining study drug will be destroyed.

9. STUDY ASSESSMENTS

9.1 Study flow chart(s) / table of study procedures and assessments

Study Periods	**Screening**		**Treatment Period (TP) 1 (placebo or verum)**			**Washout phase**	**Treatment Period (TP) 2 (crossover to placebo or verum resp.)**		
Visit	0		1	2	3		4	5	6
Time (hour, day, week)	d-28 to -1		TP1 d1	TP1 d6-8	TP1 d7	4 – 8 weeks	TP2 d1	TP2 d6-8	TP2 d7
Subject Information and Informed Consent	x								
Demographics	x								
Medical History	x								
In- /Exclusion Criteria	x								
Physical Examination	x								
Vital Signs	x		x		x		x		x
Laboratory Tests	x		x		x		x		x
Randomisation		**cold induced thermogenesis at baseline at least 5% of RMR ➔ randomization and inclusion**	x						
Measurement of cold induced thermogenesis	x				x				x
Measurement of skin temperature during cold stimulation	x				x				x
MRI of supraclavicular BAT				x				x	
Dispense Study Drug			x				x		
needle biopsy vastus lateralis muscle				x				x	
needle biopsy supraclavicular adipose tissue				x				x	
Adverse Events			x	x	x		x	x	x
Cold induced ^{18}F-FDG uptake in BAT (FDG-PET/CT)					x				x

9.2 Assessments of outcomes

9.2.1 Assessment of primary outcome

At the end of each treatment period with prednison or placebo respectively, the primary endpoint will be assessed as follows.

- Cold induced thermogenesis (CIT): Increase in energy expenditure above resting metabolic rate during warm conditions in response to a mild cold stimulus of 2 h duration; assessed by indirect calorimetry. (d7)
  Rationale: CIT in the absence of shivering reflects the thermogenic capacity of BAT and can be measured non-invasively as a functional readout.

9.2.2 Assessment of secondary outcomes

At the end of each treatment period with prednison or placebo respectively, the secondary endpoints will be assessed as follows.

- Fat content (fat fraction) of supraclavicular BAT as determined by MRI. We will use a multi-echo Dixon sequence to determine the fat and water content as well T2* of supraclavicular adipose tissue.
  Rationale: The fat-fraction of supraclavicular BAT has been shown to correlate well to the thermogenic capacity in human adults.
- Volume of supraclavicular BAT as determined by MRI
  Rationale: glucocorticoid treatment might also influence the size of BAT depots
- Change in skin temperature in response to mild cold stimulus in the following predefined locations: supraclavicular, parasternal, umbilical, mid-thigh, lower arm, finger-tip, lower leg, back of foot. (d7)
  Rationale: Decrease in skin temperature gives information about the body's isolative response to mild cold exposure.
- Supraclavicular skin temperature in response to mild cold stimulus
  Rationale: SC skin temperature has been described as an easily measurable surrogate parameter of the scBAT depot activity.
- Volume of supraclavicular BAT as determined by MRI
- At the end of each treatment period with prednisone or placebo respectively, ^{18}F-FDG-PET CT uptake into BAT after 2.0 h of cold stimulation will be assessed. It will be reported according to the recently defined "Brown Adipose Reporting Criteria in Imaging Studies" (BARCIST 1.0)[35].
  Supraclavicular BAT activity will be quantified as mean standardized uptake value (SUV_{mean}) in a fixed volume of interest in the supraclavicular adipose tissue region.*Rationale:* Currently, cold stimulated ^{18}F-FDG uptake into adipose tissue is widely used as a reliable imaging parameter of BAT function[36].

9.2.3 Assessment of other outcomes of interest

- Levels of FGF21 in plasma
  Rationale: FGF21 has been shown to be associated with BAT activity in humans.
- Plasma glucose and insulin level before and after cold stimulation
- LDL cholesterol and triglyceride levels in plasma before and after cold stimulation at the end of the respective study period.
  Rationale: Active BAT has been shown to actively take up glucose and plasma lipids in response to cold exposure independently of insulin signalling.
- Gene and protein expression in supraclavicular adipose tissue: specifically UCP1, DIO2, PGC1α, PPARγ, PRDM16, 11-β-HSD1, CKMT1
  Rationale: we will take subcutaneous adipose tissue biopsies to investigate the effects of GC medication on white adipose tissue "browning" on a molecular level. Within the subcutaneous adipose tissue depot so called beige adipocytes can emerge.
- Gene and protein expression in muscle: SERCA 1 and 2, sarcolipin.
  Rationale: Muscle can contribute to non-shivering thermogenesis by futile calcium cycling. We want to study the effects of GC medication on key effectors of calcium cycling.

9.2.4 Assessment of safety outcomes

9.2.4.1 Adverse events

The occurrence of an AE will be determined based on observed or volunteered signs and symptoms, as well as changes in the participant's physical examination and laboratory results. AEs that occur during the study will be recorded on the appropriate Adverse Event pages of the eCRF.

If participants report side effects, the investigator decides whether the study can continue or the subject should be excluded from the study.

9.2.4.2 Laboratory parameters

The following parameters will be analysed in plasma at study visit 1, 3 and 4, 6.

- Fasting serum glucose
- Alanine amino transferase (ALAT)
- Aspartate amino transferase (ASAT)

The analysis will be performed at the central lab of the university hospital Basel. An increase of ASAT/ALAT above 3x ULN will be defined as adverse event.

9.2.4.3 Vital signs

The following vital signs will be assessed at each study visit after at least 5 min of rest

- Heart rate (pulse), by electronic blood pressure meter; limits: 45 to 100 bpm
- Blood pressure, by electronic blood pressure meter; limits: systolic 90 to 160 mmHg
- Core temperature, by infrared ear thermometer; limits: 36.0 to 37.8 °C.

9.2.5 Assessments in participants who prematurely stop the study

Participants who are withdrawn from the study prematurely are contacted by phone to assess adverse events of trial medication within 2 weeks after the last study day.

9.3 Procedures at each visit

9.3.1 Screening visit (Visit 0, at least 1 d and maximum 28 days before visit 1)

Interested volunteers will be contacted by telephone call or email. Eligibility criteria will be evaluated. If study demands are fulfilled, an appointment for the screening visit will be arranged. Study information will be explained to the prospective participants.

Demographics, medical history and vital signs will be recorded and eligibility criteria checked.

The following procedures are to be performed at the screening visit

- Informed consent is signed
- Baseline lab sample is drawn (complete blood count, serum creatinine, serum potassium, TSH, free T4, lipid profile, glycated haemoglobin A1c, INR)
- Cold induced thermogenesis is measured as follows:
    - Participants will rest on a bed for 30 minutes before the first measurement of REE.
    - During the first measurement of REE during (warm conditions) the subject wearing a T-Shirt and underwear will be covered by a duvet.
    - After the measurement during warm conditions water-circulating cooling/warming sleeves connected to a medical cooling device (Hilotherm Clinic®, Hilotherm GmbH, Germany) will be placed around the subject's abdomen and lower back and the duvet will be removed. Initially the temperature of the water will be set to 25 C [22]. A mild cold stimulus will be applied by reducing the temperature of the circulating water by approximately 1 C every 2 minutes to a minimum of 10 C. If the participant feels uncomfortably cold or starts to shiver reduction of the temperature will be stopped and the temperature will be elevated by 2 C to prevent shivering (cooling protocol adapted from (REF)). Cooling will be carried out for a total of 120 minutes. The energy expenditure will be measured again during the final 30 min by indirect calorimetry with the water temperature in the cooling sleeves at a constant temperature.
    - In parallel to the assessment of energy expenditure, supraclavicular, infraclavicular, abdominal and mid-thigh skin temperature will be assessed non-invasively by Thermochrom iButtons taped to the skin at 1 minute intervals. Body core temperature

will be measured with an infrared ear thermometer.
    - blood pressure and heart rate will be measured during the cooling procedure at predefined time points.
- If the participant shows at least 5% increase of energy expenditure above REE at warm temperature in response to the cold stimulus, he may proceed to the treatment phase of the study. Otherwise the participant will not continue in the study.
- If the participant does not fulfil the eligibility criteria he will not continue into the study.

9.3.2 Visit 1 (TP 1 / day 1)

The following procedures are performed

- Randomization is performed using the Secutrial system.
- Vital signs recorded
- Patient asked for adverse events: abdominal problems, other adverse events
- Lab sample is drawn: ASAT, ALAT, cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, fasting plasma glucose, fasting plasma insulin, FGF21, INR, complete blood count.
- First dose of study medication or placebo, respectively, is dispensed
- Study medication is handed over to the participant

9.3.3 Visit 2 (TP 1 / day 6-8)

The following procedures are performed

- Magnetic resonance imaging of the supraclavicular adipose tissue depot.

After MRI tissue biopsies will be taken from the supraclavicular adipose tissue and the vastus lateralis muscle. Biopsies will be performed by a trained physician using ultrasound guidance.

- Supraclavicular adipose tissue:
    - Localisation of the puncture site using the MRI images and anatomic landmarks.
    - Thorough disinfection of the skin with alcohol based antiseptic and placement of sterile drape
    - Local anesthesia with lidocaine 1%
    - The skin is punctured with the Bard TruGuide® disposable co-axial Needle (13 Gauge, 2.4 mm diameter).
    - The Bard Max-Core® biopsy needle with 14 Gauge (2.2 mm Diameter) is introduced through the introducer needle. Two core biopsies (22 mm length) are taken from the muscle by releasing the automatic biopsy mechanism.
    - The biopsy needle is removed and local pressure applied to prevent bleeding.
- Vastus lateralis muscle [37]:
    - The puncture site on the lateral thigh is marked, disinfected and covered by a sterile drape with central hole.
    - Local anesthesia with 1% lidocaine
    - The skin is punctured with the Bard TruGuide® disposable co-axial Needle (13 Gauge, 2.4 mm diameter) and the needle advanced to the fascia of the muscle.
    - The Bard Max-Core® biopsy needle with 14 Gauge (2.2 mm Diameter) is introduced through the introducer needle. Two core biopsies (22 mm length) are taken from the muscle by releasing the automatic biopsy mechanism.
    - The biopsy needle is removed and local pressure applied to prevent bleeding.

9.3.4 Visit 3 (TP 1 / day 7)

The following procedures are performed

- Vital signs recorded
- Patient asked for adverse events: abdominal problems, other adverse events
- Second dose of study medication or placebo, respectively, is dispensed
- Cold induced thermogenesis will be measured as follows:
    - Participants will rest on a bed for 30 minutes before the first measurement of REE.
    - During the first measurement of REE during (warm conditions) the subject wearing a T-Shirt and underwear will be covered by a duvet.

- A peripheral intravenous line is placed.
- Blood sample is drawn: plasma glucose, serum insulin, serum triglycerides, serum cholesterol and FGF21.
- After the measurement during warm conditions water-circulating cooling/warming sleeves connected to a medical cooling device (Hilotherm Clinic®, Hilotherm GmbH, Germany) will be placed around the subject's abdomen and lower back and the duvet will be removed. Initially the temperature of the water will be set to 25 C. A mild cold stimulus will be applied by reducing the temperature of the circulating water by approximately 1 C every 2 minutes to a minimum of 10 C. If the participant feels uncomfortably cold or starts to shiver reduction of the temperature will be stopped and the temperature will be elevated stepwise by 1 C to stop shivering. Cooling will be carried out for a total of 120 minutes. The energy expenditure will be measured again after 90 min of cooling for 30 min by indirect calorimetry with the water temperature in the cooling sleeves at a constant temperature.
- Blood sample is drawn at the end of the cooling phase: plasma glucose, serum insulin, serum triglycerides, serum cholesterol and FGF21.
- In parallel to the assessment of energy expenditure, supraclavicular, infraclavicular, abdominal and mid-thigh skin temperature will be assessed non-invasively by Thermochrom iButtons taped to the skin at 1 minute intervals. Body core temperature will be measured with an infrared ear thermometer.

PET/CT-scanning

- Capillary glucose prior to FGD-PET in order to avoid hyperglykemia which would interfere with the PET measurement
- Cold induced glucose uptake into BAT and cold induced thermogenesis will be measured as follows (see also Figure 3).
  - Immediately after the second calorimetry participants will be transferred to the PET/CT scanning room.
  - 75 MBq of 18-Fluoro-Deoxy-Glucose (^{18}F-FDG) are injected through the intravenous catheter.
  - Dynamic PET scanning of the neck-region will be performed during 30 minutes. Thereafter static PET scanning will be performed for another 10 minutes (2 bed-positions)
  - Glucose uptake into BAT will be calculated according to standard methods.

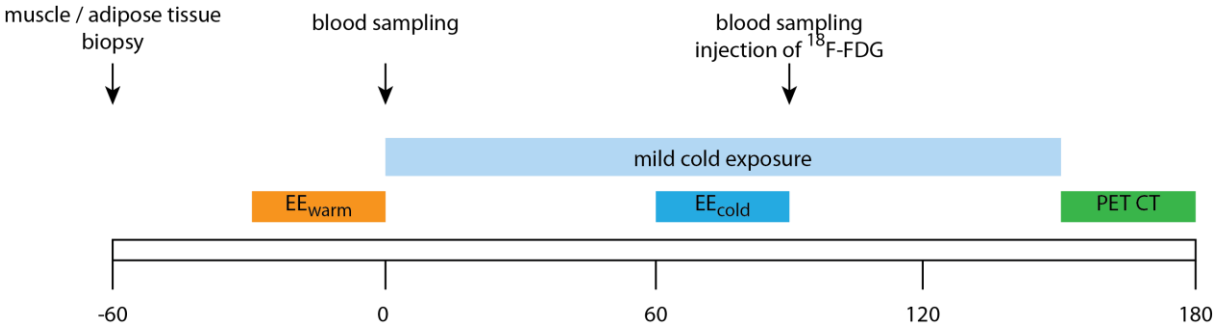

Figure 2: Procedures during study visit 3.

9.3.5 Washout phase

There will be a washout phase of at least 4 weeks, maximum 8 weeks.

9.3.6 Visit 4 (TP 2 / day 1)

The procedures are analogous to visit 1

9.3.7 Visit 5 (TP 2 / day 6-8)

The procedures are analogous to visit 2

9.3.8 Visit 6 (TP 2 / day 7)

The procedures are analogous to visit 3

10. SAFETY

10.1 Drug studies

During the entire duration of the study, all adverse events (AE) and all serious adverse events (SAEs) are collected, fully investigated and documented in source documents and the eCRF. Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period.

10.1.1 Definition and assessment of (serious) adverse events and other safety related events

An **Adverse Event (AE)** is any untoward medical occurrence in a patient or a clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with the study procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. [ICH E6 1.2]

A **Serious Adverse Event (SAE)** is classified as any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires in-patient hospitalization or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]

SAEs should be followed until resolution or stabilisation. Participants with ongoing SAEs at study termination (including safety visit) will be further followed up until recovery or until stabilisation of the disease after termination.

Assessment of Causality

Both Investigator and Sponsor-investigator make a causality assessment of the event to the study drug, based on the criteria listed in the ICH E2A guidelines:

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	

Unexpected Adverse Drug Reaction

An “unexpected” adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator’s Brochure for drugs that are not yet approved and Product Information for approved drugs, respectively). [ICH E2A]

Suspected Unexpected Serious Adverse Reactions (SUSARs)

The Sponsor-Investigator evaluates any SAE that has been reported regarding seriousness, causality and expectedness. If the event is related to the investigational product and is both serious and unexpected, it is classified as a SUSAR.

Assessment of Severity

For this study the severity grading scale “Common Terminology Criteria for Adverse Events CTCAE Version 4.0” is used.

10.1.2 Reporting of serious adverse events (SAE) and other safety related events

Reporting of SAEs

All SAEs must be reported immediately and within a maximum of 24 hours to the Sponsor-Investigator of the study. The Sponsor-Investigator will re-evaluate the SAE and return the form to the site.

SAEs resulting in death are reported to the local Ethics Committee (via local Investigator) within 7 days.

Reporting of SUSARs

A SUSAR needs to be reported to the local Ethics Committee (local event via local Investigator) and to Swissmedic for category B and C studies (via Sponsor-Investigator) within 7 days, if the event is fatal, or within 15 days (all other events).

Reporting of Safety Signals

All suspected new risks and relevant new aspects of known adverse reactions that require safety-related measures, i.e. so called safety signals, must be reported to the Sponsor-Investigator within 24 hours. The Sponsor-Investigator must report the safety signals within 7 days to the local Ethics Committee (local event via local Investigator) and to Swissmedic in case of a category B or C study.

Periodic reporting of safety

An annual safety report is submitted once a year to the local Ethics Committee via local Investigator and to Swissmedic.

10.1.3 Follow up of (Serious) Adverse Events

Following a comprehensive baseline evaluation, each subject’s safety will be monitored with periodic recording and evaluation of all treatment-emergent AEs.

Once evidence of a clinical abnormality is noticed, the condition will be treated while trying to determine its cause. The subject will then be followed until the condition resolves or becomes chronic or stable. Patients will be instructed about possible acute AEs. If adverse events are observed, the participants are promptly treated according to standard of care.

10.1.4 Safety aspects associated with ionizing radiation exposure due to PET/CT imaging

In order to reduce the radiation exposure for participants in this study the following precautions and measures will be taken.

As the main human BAT depot is in the supraclavicular region, we will only perform low-dose CT scanning of this region (neck and upper thorax aperture). This region can be covered with 2 bed-positions of the scanner with the region of main interest covered by the overlapping scan region (total scan length 24 cm)

The amount of ^{18}F-FDG applied will be considerably lower than for routine clinical scans and has also been used in other studies of human BAT[25, 38]. To compensate for the lower amount of FDG the acquisition time per bed position will be increased to 10 min.

With these parameters the calculation of the radiation dose per scan is calculated as follows:

Dose due to topogramm: 0.29 [CTDI] x 32 cm x 0.01 = 0.093 mSv

Dose due to CT scan (2 bed positions, each 16 cm, overlapping 8 cm, resulting in 24 cm scan length)

2.70 [CTDI] x 24 cm x 0.01 = 0.65 mSVv

Dose due to 75 MBq ^{18}F-FDG: 1.44 mSv

Total dose per scan (rounded to 0.1 mSV): 2.2 mSv

Total dose per participant for complete study: 4.4 mSv.

This will be below the threshold of 5 mSv deemed low risk according to ClinO Art 2.

10.1.5 Safety aspects associated with taking biopsies from supraclavicular adipose tissue and lateral thigh muscle (vastus lateralis muscle)

Needle biopsies of supraclavicular adipose tissue and vastus lateralis muscle are widely used in clinical research. They are less invasive than open surgical biopsies or use of the traditional 6 mm diameter Bergström needle technique.

However, the following complications may occur: bleeding at the puncture site, lesion to nerves, infection, and pneumothorax. The puncture sites are situated in locations with a low density of vessels and nerves, so the risk of compliations is deemed low. In order to avoid infection, the skin will be disinfected thoroughly and aseptic technique be used.

11. STATISTICAL METHODS

11.1 Hypothesis

Based on in vitro studies in human cell culture and in vivo studies in mice, we hypothesize that GCs reduce the activity of BAT in humans.

The null hypothesis is that there will be no significant difference between cold induced thermogenesis after a treatment with placebo versus prednisone.

11.2 Determination of Sample Size

For calculation of sample size the following assumptions were made:

Based on in vitro studies and in vivo studies in mice we expect a substantial effect of prednisone on BAT thermogenesis (reduction of cold induced thermogenesis by at least 33%). In order to be able to detect a reduction in CIT, we will only include participants which exhibited a significant amount of CIT of at least 5% of REE.

The power of the trial (1- type two error) was set to 80%, the type one error level to 5%. Analysis will be two-tailed using a Wilcoxon-signed rank test for matched pairs (not assuming normal distribution of the primary endpoint). Based on prior studies we expect a correlation between the two measurements in each subject of 0.7.

Using these assumptions, we calculate a minimal sample size of 10 participants to detect a significant difference. In order to account for heterogeneity of the sample and dropouts we aim to include 16 participants into the study. This will allow us to detect a clinically meaningful difference in BAT function in response to cold exposure with a power of > 85%. Sample size calculation was performed with G*Power software Version 3.1.9, University of Düsseldorf.

11.3 Statistical criteria of termination of trial

None defined.

11.4 Planned Analyses

The statistical analyses will be performed with the R statistical package (R foundation, Vienna, Austria).

11.4.1 Datasets to be analysed, analysis populations

The full analysis set (FAS) will include all randomised subjects. The complete analysis set (CAS) will include only those patients who completed both study arms. Demographics and baseline characteristics of participants as well as number of drop-outs will be reported for the CAS. All statistical analyses will be performed on the CAS.

11.4.2 Primary Analysis

The primary analysis will be performed after all participants have completed the trial.

The difference in cold induced thermogenesis will be analysed by Wilcoxon signed rank-test (matched pairs). α error probability will be set to 0.05.

11.4.3 Secondary Analyses

The secondary analyses will be performed after all participants have completed the trial.

Not assuming normal distribution of the outcomes pairwise comparison will be performed by Wilcoxon signed rank tests.

Specifically the following outcomes will be analysed (prednisone vs. placebo):

- MRI determined fat fraction of supraclavicular BAT
- MRI determined volume of supraclavicular BAT
- cold stimulated glucose uptake into supraclavicular BAT determined as SUV_{mean} in two volumes of interest in the supraclavicular adipose tissue
- SUV_{max} in the supraclavicular adipose tissue depot
- Cold induced thermogenesis: Increase in energy expenditure above resting metabolic rate in response to a mild cold stimulus.
- Supraclavicular (SC) skin temperature in response to mild cold stimulus
- Delta SC skin temperature cold vs. warm condition
- Glucose and insulin level before and after mild cold stimulus
- Delta FGF21 level before and after mild cold stimulus
- Delta finger-tip temperature in response to mild cold stimulus as a measure of peripheral vasoconstriction.
- LDL and triglyceride levels after each treatment period

11.4.4 Interim analyses

Interim analyses are not planned.

11.4.5 Safety analysis

A safety analysis does not apply, as prednisone is already approved as drug by Swissmedic.

11.4.6 Deviation(s) from the original statistical plan

If substantial deviations of the analysis as outlined in this sections are needed for whatever reason the protocol will be amended. All deviations of the analysis from the protocol or from the detailed analysis plan will be listed and justified in a separate section of the final statistical report.

11.5 Handling of missing data and drop-outs

Careful trial planning and conducting will minimise the occurrence of missing data as far as possible. No imputation of missing data is planned. Drop outs will be replaced.

12. QUALITY ASSURANCE AND CONTROL

12.1 Data handling and record keeping / archiving

12.1.1 Case Report Forms

The clinical data will be recorded in the electronic Case Report Forms (eCRF) in an encrypted fashion by their individual study participant number and will be collected in an electronic data capture (EDC) system, named secuTrial®. The EDC system runs on a server maintained by the IT-department of the University Hospital Basel. The electronic CRF (eCRF) is implemented (set-up and adjusted) by the data management group at the Clinical Trial Unit (CTU) at the University Hospital Basel.

Data will be entered into the eCRF by investigators and study nurses.

12.1.2 Specification of source documents

- Demographic data of participants
- Study number
- Visit dates
- Results of laboratory analysis
- Results of indirect calorimetry measurements
- Results of PET-CT scans
- Results of skin temperature monitoring

The source data will be stored at the Department of Endocrinology, Diabetes and Metabolism, University Hospital Basel.

12.1.3 Record keeping / archiving

Records and documents pertaining to the conduct of this study, including CRFs, consent forms, laboratory test results and imaging data, will be stored at the Department of Endocrinology, Diabetes and Metabolism, University Hospital Basel, for 10 years after completion of the study.

12.2 Data management

12.2.1 Data Management System

The clinical data will be collected in an electronic data capture (EDC) system, named secuTrial®. The EDC system runs on a server maintained by the IT-department of the University Hospital Basel. The electronic CRF (eCRF) is implemented (set-up and adjusted) by the data management group at the Clinical Trial Unit (CTU) at the University Hospital Basel.

12.2.2 Data security, access and back-up

The EDC system is accessible via a standard browser on a WWW-connected device.

Password protection ensures that only authorized persons can enter the system to view, add or edit data according to their permissions. User administration and user training is performed by the CTU according to predefined processes. Back-up of secuTrial® study data is performed according to the processes of the IT-department of the University Hospital Basel.

12.2.3 Analysis and archiving

The EDC will be locked after all data has been monitored and all raised queries have been resolved. Data is exported and transferred to the investigator by the CTU according to internally defined processes. Data will be archived by the investigator.

12.2.4 Electronic and central data validation

Data is entered into the eCRF and can be validated for completeness and discrepancies automatically. An audit trail system maintains a record of initial entries and changes (reasons for changes, time and clinical study protocol date of changes, user identification of entry and changes).

12.3 Monitoring

Data will be entered into the eCRF and can be validated for completeness and discrepancies automatically. An audit trail system maintains a record of initial entries and changes (reasons for changes, time and date of changes, user identification of entry and changes).

The study will be monitored by the Clinical Trials Unit (CTU) of the University Hospital Basel. The source data will be accessible to monitors and questions will be answered by the principal investigator during monitoring. Monitoring visits are planned prior to inclusion of the first participant and after the inclusion of the first participant as well as after every fourth participant. The monitoring will comprise informed consent forms, source data and CRFs of all participants.

12.4 Audits and Inspections

Authorized representatives of the national or local authorities will be permitted to inspect or audit the facilities and records relevant to this study. All involved parties must keep the participant data strictly confidential.

12.5 Confidentiality, Data Protection

All personal and medical information obtained for this study is confidential and disclosure to third parties other than those noted below is prohibited. Patients' data will be identified by study and subject ID number.

Confidentiality of the patients will be maintained by assigning patients a study number, keeping identifiers separate from the data and storing data in a locked file in the department of Endocrinology, Diabetes and Metabolism. The sponsor investigator, the principle investigator, co-investigators and the study nurses will have access to the encryption list.

Scientific reports generated from the study will not contain information that would identify the participant. After termination of the study records will be archived for ten years and then destroyed.

Upon the participant's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for her or his welfare.

12.6 Storage of biological material and related health data

Biological material, i.e. blood samples, will be handled according to good clinical practice and good laboratory practice. Samples will be collected in secure containers (Sarstedt Monovette®) and will be centrifuged to collect serum. Serum will be stored at – 80°C in a thermo-controlled ultra-deep freezer.

Data and samples are stored for eventual future research aims. Records and documents pertaining the conduct of this study, including eCRFs, consent forms, laboratory test results and clinical notes will be retained for 10 years.

13. PUBLICATION AND DISSEMINATION POLICY

The results of this study shall be published in a peer-reviewed journal and presented at scientific conferences. The decision where and when to publish the results of the study is entirely up to the investigators and not restricted by thirds parties. All investigators participating in this study will be eligible for authorship.

14. FUNDING AND SUPPORT

14.1 Funding

Study costs will be covered by a Swiss National Science Foundation grant to Matthias Betz. Additionally, funding is sought from the Bangerter-Rhyner-Foundation and the Wissenschaftspool of the University Hospital Basel.

14.2 Other Support

The Department of Endocrinology, Diabetes and Metabolism, University Hospital Basel, will provide the location and infrastructure.

15. INSURANCE

Insurance will be provided by the Department of Endocrinology, Diabetes and Metabolism of University Hospital of Basel. A copy of the certificate is filed in each investigator site file and the trial master file.

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17. APPENDICES

- List of parameters for eCRF
- Participant information and informed consent