BMJ Open

Rationale and Design of the CORE (COrticosteroids REvised) study: A Randomized Cross-over Clinical Trial of Prednisolone versus Dexamethasone

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-061678
Article Type:	Protocol
Date Submitted by the Author:	02-Feb-2022
Complete List of Authors:	Stam, Suzanne; University Medical Centre Groningen, Internal Medicine, division of Nephrology Vulto, Annet; University Medical Centre Groningen, Internal Medicine, division of Endocrinology Vos, Michel; University Medical Centre Groningen, Laboratory Medicine Kerstens, Michiel; University Medical Centre Groningen, Internal Medicine, division of Endocrinology Rutgers, Abraham; University Medical Centre Groningen, Rheumatology and Clinical Immunology Kema, Ido; University Medical Centre Groningen, Laboratory Medicine Touw, Daan; University Medical Centre Groningen, Clincal Pharmacy and Pharmacology Bakker, Stephan; University Medical Centre Groningen, Internal Medicine, division of Nephrology van Beek, André; University Medical Centre Groningen, Internal Medicine, division of Endocrinology
Keywords:	General endocrinology < DIABETES & ENDOCRINOLOGY, Sex steroids & HRT < DIABETES & ENDOCRINOLOGY, CLINICAL PHARMACOLOGY, INTERNAL MEDICINE

Rationale and Design of the CORE (COrticosteroids REvised) study: A Randomized Cross-over Clinical Trial of Prednisolone versus Dexamethasone

Suzanne P. Stam, MD[1]*, Annet Vulto, MD[2]*, Michel J. Vos, PhD[3], Michiel N. Kerstens, MD,PhD[2], Abraham Rutgers, MD, PhD[4], Ido P. Kema, PhD[3], Daan J. Touw, PharmD, PhD[5], Stephan J.L. Bakker MD,PhD[1], André P. van Beek, MD,PhD[2]

* contributed equally

[1] Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[2] Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[3] Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands

[4] Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[5] Department of Clinical Pharmacy and Pharmacology, University of Groningen, Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands

Running title:

Randomized clinical trial on Prednisolone and Dexamethasone Bio-equivalency

Names for PubMed Indexing:

Stam SP, Vulto A, Vos MJ, Kerstens MN, Rutgers A, Kema IP, Touw DJ, Bakker SJL, Van Beek AP

Word count: 3998 (max 4000 words) Abstract words: 225 words

Tables: 3 Figure: 1

Corresponding author:

A.P. van Beek, MD PhD

Department of Internal Medicine, Division of Endocrinology

University Medical Center Groningen

P.O. Box 30.001, 9700 RB Groningen, the Netherlands

Phone: 0031 050 361 0388, Email: a.p.van.beek@umcg.nl

Administrative information

Title {1}	Rationale and Design of the CORE (COrticosteroids REvised) study: A Randomized Cross-over Clinical Trial of Prednisolone and Dexamethasone
Trial registration {2a and 2b}.	EudraCT: 2019-004983-23 ClinicalTrial.gov: NCT04733144 Dutch trial registry (NTR): NL9138
Protocol version {3}	3.0; date 23rd December 2020
Funding {4}	Investigator-initiated
Author details {5a}	Suzanne P. Stam: Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. Annet Vulto: Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. Michel J. Vos: Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands Michiel N. Kerstens: Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. Abraham Rutgers: Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. Ido P. Kema: Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

	Daan J. Touw: Department of Clinical Pharmacy and Pharmacology, University of Groningen, Groningen, University Medical Center Groningen, Groningen, The Netherlands Stephan J.L. Bakker: Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. André P. van Beek: Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.
Name and contact information for the trial sponsor {5b}	University Medical Center Groningen Hanzeplein 1, Postbus 30001, 9700 RB Groningen, The Netherlands
Role of sponsor {5c}	N.A.

Abbreviations

AUC	-	Area under the curve
CBG	-	Cortisol binding globulin
GLP-1	-	Glucagon-like peptide-1
GR	-	Glucocorticoid receptor
HPA-axis	-	Hypothalamic pituitary adrenal axis
LC-MS/MS	-	Liquid chromatography–tandem mass spectrometry
MR	-	Mineralocorticoid receptor
SAE	-	Serious adverse event
UMCG	-	University Medical Center Groningen

Abstract

Introduction. Corticosteroids are an important pillar in many anti-inflammatory and immunosuppressive treatment regimens and are available in natural and synthetic forms, which are considered equipotent if bio-equivalence data are utilized. Current bio-equivalence data are however based on animal studies or studies with subjective endpoints. Furthermore, advancement in steroid physiology with regard to metabolization, intracellular handling and receptor activation have not yet been incorporated. Therefore, this study aims to re-examine the bio-equivalence and dose effects of the most widely used synthetic corticosteroids, prednisolone and dexamethasone.

Methods. In this double-blind, randomized cross-over clinical trial, 24 healthy male and female volunteers aged 18-75 years, will be included. All volunteers will randomly receive either first a daily dose of 7.5 mg prednisolone for one week, immediately followed by a daily dose of 30 mg prednisolone for one week, or first a presumed bio-equivalent dose of 1.125 mg dexamethasone per day, immediately followed by 4.5 mg of dexamethasone per day for one week. After a 4–8 week wash-out period the other treatment will be applied. The primary study endpoint is the difference in total endogenous cortisol excretion in 24h urine. Secondary endpoints will include differences in immunological parameters, blood pressure, and metabolic measurements.

Ethics and dissemination. This study has been approved by the Medical Ethics Committee of the University Medical Center Groningen (METC 2020.398) and registered on ClinicalTrials.gov (NCT04733144).

Keywords

Corticosteroids, Prednisolone, Dexamethasone, bio-equivalence, healthy subjects

Strengths and Limitations

1. Cross-over design limits high inter-individual effect of exogenous glucocorticoids
2. State-of-the-art laboratory techniques
3. Utilized doses reflect clinical practice
4. Absence of placebo intervention
5. Due to the COVID-19 pandemic and subsequent vaccination campaign wash-out could not always be maintained at 4 to 8 weeks.

Introduction

Since the first clinical use of cortisone in 1948, corticosteroids have become a fundamental part in the treatment of many diseases, including autoimmune disorders, respiratory disorders, and haematological malignancies [1]. Furthermore, corticosteroids have become a mainstay in the immunosuppressive treatment for solid organ transplantation. Corticosteroids are available in various natural and synthetic forms [2]. In a clinical setting, different natural and synthetic forms are applied interchangeably, for which equipotent doses can be calculated according to established bio-equivalence data [3]. Although this is more or less thoughtlessly applied in daily practice, it is important to realise that the literature which provides the rationale for the current bio-equivalence data, consists of non-randomized studies carried out in the fifties [4,5]. In addition, these studies are limited by the use of subjective endpoints, outdated laboratory techniques, and the use of animals or patients with rheumatoid arthritis as study participants [4–6]. In the seventies some attempts have been made to improve bio-equivalence data of corticosteroids, but these attempts were hampered by methodological imperfections [7–9]. Since then one pharmacological study, performed approximately twenty years ago, suggested that the current dosing tables reflect a reasonable dose equivalence relation, but this study included only 5 men and described only the effects of a single interventional dose [10]. Furthermore, recent decades have resulted in major advancements in our knowledge of corticosteroids, especially on intracellular handling and receptor transactivation or –repression but this has not yet resulted in a better understanding of their bioequivalence.

Predniso(lo)ne and dexamethasone are the most commonly prescribed representants of the synthetic corticosteroids and therefore provide an important focus to study bioequivalence. When studying this, effects on the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), metabolization or intracellular handling as well as tissue or system specific transactivation or -repression should be taken into account. Regarding the first, predniso(lo)ne

and dexamethasone have divergent effects, because whilst both have GR effects (i.e. anti-inflammatory and immunosuppressive properties), only predniso(lo)ne has MR effects [11]. Although these characteristics are known since their discovery, it may have important consequences for various organ systems relying on mineralocorticoid effects such as the brain and kidney, resulting in different (side) effects. Novel insights have also unveiled a difference in metabolization, for example due to an alternative intracellular handling by both 11β-hydroxysteroid dehydrogenase type 1 and type 2 [12]. It can therefore be hypothesized that currently presumed equipotent doses of prednisolone and dexamethasone have different effects on various organ systems for which these enzymes are important. Also, advancement in the understanding of the molecular mechanism of the GR has uncovered a wide range of system specific sensitivities to corticosteroids [13,14]. This indicates that the currently used approach of one conversion factor for all body systems may not be justified. Instead, it may be necessary to take this heterogenicity into account, by utilizing system specific conversion rates. Finally, as studies have demonstrated that the pharmacokinetics of prednisolone are non-linear, whilst those of dexamethasone are, it may be postulated that the conversion factor between prednisolone and dexamethasone is dose-dependent [15].

Therefore, we aim to re-examine the bio-equivalence and dosing effects of prednisolone and dexamethasone on various physiological systems, to provide reliable in vivo data in healthy volunteers and thus provide data to optimize systemic corticosteroid therapy to modern day standards.

Methods

Study design

The CORE study is an investigator-initiated, single-center, randomized, double-blind, crossover trial including healthy volunteers to receive two doses of prednisolone and two doses of dexamethasone. All volunteers will be randomly assigned to receive either first a daily dose of 7.5 mg prednisolone for one week, immediately followed by a daily dose of 30 mg of prednisolone for one week, or first a presumed bio-equivalent dose of 1.125 mg dexamethasone per day, immediately followed by 4.5 mg of dexamethasone per day for one week (figure 1). After a four to eight week wash-out period the other treatment will be applied. The duration of the wash-out period is at least four weeks, but can be extended to eight weeks to prevent the influence of stressful periods such as exams and work deadlines. The primary outcomes of the trial is the difference in 24h urinary total cortisol excretion between lowest doses and highest doses of prednisolone and dexamethasone.

Study setting and population

All study visits will be performed in the outpatient clinic of the University Medical Center Groningen (UMCG), an academic hospital in the northern part of the Netherlands. A total of 24 healthy volunteers will be included in the study. As most of the outcomes are dependent on age and sex, the participants are subdivided into 4 groups, specifically 6 males aged 18-50 years, 6 females aged 18-50 years and using oral contraceptives, 6 males aged ≥50-75 years, and lastly 6 postmenopausal females aged ≥50-75 years. Next to the age and hormonal status mentioned above, volunteers need to have a BMI between 18.5-30 kg/m^2, no relevant medical history, and no dependency on any type of corticosteroid in any pharmaceutical form. All inclusion and exclusion criteria can be found in table 1. Participants will either be recruited through pamphlets placed in local public buildings or advertisement in the local newspaper.

Table 1. Inclusion and exclusion criteria for the CORE study

Inclusion criteria	Exclusion criteria
1. Participants must have good command of the Dutch language 2. Participants must provide written informed consent 3. Participants must have an age between 18 – 75 years old 4. Female participants aged 18-49 years must be using oral contraceptives and female participants age 50-75 years must be in the postmenopausal state 5. BMI between 18.5 and 30 kg/m2 6. Participants are not allowed to have a relevant medical history or use interfering medication	1. Potential participants with a medical history of: a. Diseases affecting the HPA-axis: e.g. primary and secondary adrenal insufficiency, pituitary tumors, or Cushings' disease b Chronic inflammatory diseases: e.g. rheumatoid arthritis, polymyalgia rheumatica, and asthma c. Psychiatric diseases d. Diabetes mellitus 2. Potential participants who have known contraindication to the study medication (e.g. known peptic ulcer disease or active infectious disease) 3. Night shift workers 4. Potential participants with a kidney function <60 ml/min/1.73m2, abnormalities in liver enzymes, and/or abnormalities in thyroid function 5. Potential participants who are dependent on corticosteroids in any form, e.g. asthmatic patients, and transplant recipients 6. Potential participants who utilize any medication which is likely to confound assessment of one the endpoints (e.g. inhaled corticosteroids, hormone supplements, psychotropic drugs, carbamazepine or vaccination) 7. Potential participants who intend to undergo significant lifestyle changes e.g. voluntary weight loss and discontinue smoking habits. 8. Potential participants who are unlikely to adhere to the study medication (e.g. volunteers with a history of substance abuse or non-adherence)

Ethics

The CORE study is conducted according to the principles of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO, The Netherlands). The current study has been approved by the Medical Ethical Committee of the UMCG, The Netherlands (METC 2020.398) on the 18th of January 2021, has been registered on ClinicalTrials.gov (Identifier: NCT04733144), and in the Dutch trial registry (NL9138). Potential protocol amendments will be submitted to the Medical Ethical Committee for review and subsequently distributed to volunteers. Potential participants need to actively seek contact with the investigators and when interested will receive written information. Prior to obtaining informed consent, research staff will explain the aim of the study and all study procedures to the volunteers. Additionally, the research staff will explain that participation is voluntary and that participants are able to withdraw their consent at any given point in time. If the potential participant has no further questions, written informed consent will be obtained from all volunteers by a study physician (SS or AV). Simultaneously, participants are asked if collected data may be used for ancillary studies and if in agreement provide written informed consent. Participants will receive a financial compensation of € 500,-. A full SPIRIT statement checklist can be found in the supplemental material.

Patient and Public Involvement

As this study is performed with healthy subjects, patients were not directly involved to the design of the study. Recruitment of participants was however updated based on input of the volunteers.

Intervention

This study is designed as a crossover trial as previous studies have demonstrated a high inter-individual variation for exogenous corticosteroids [16,17]. One intervention consists of two doses of prednisolone (11β,17,21-trihydroxy-1,4-pregnadieen-3,20-dion). To align the CORE study as much as possible with current clinical practice, the doses that were chosen were based on dosages which are often prescribed in clinical practice. In general, a distinction is made between maintenance doses, ranging from 5-20 mg prednisolone daily and active treatment doses, ranging from 30-80 mg prednisolone daily. To minimize potential side effects, we selected a low maintenance dose at a borderline physiological level, namely 7.5 mg prednisolone and a low active treatment dose namely 30 mg prednisolone, both for the duration of a week. To allow for comparison between prednisolone and dexamethasone, the currently presumed bio-equivalency data of dexamethasone (9-fluor-11β,17,21-trihydroxy-16α-methyl-1,4-pregnadieen-3,20-dion) were used, resulting in 1.125 mg dexamethasone and 4.5 mg dexamethasone, respectively [18]. All study medication was taken every day at eight o'clock in the morning after an overnight fast and provided to participants as capsules for oral ingestion. No tapering is applied as both intervention periods are no longer than two weeks [19]. To monitor interventional adherence, all remain drug capsules were counted upon return during the study visit.

Primary outcome

<u>24h urinary cortisol excretion</u>

The primary composite endpoint is the difference between the two lower doses and two higher doses of prednisolone and dexamethasone measured by 24h urinary total cortisol excretion as measure for hypothalamic-pituitary-adrenal axis (HPA-axis) suppression (24h cortisol $Pred_{7.5mg}$ – $Dex_{1.125mg}$ and 24h cortisol $Pred_{30mg}$ – $Dex_{4.5mg}$). For this endpoint, 24h urine is collected

according to a strict protocol which is as follows: on the morning of the day before a study visit, participants are asked to discard a urine void and subsequently collect all urine for the next 24h including a urine void at exactly 24h after the first discarded urine void. Next to 24h urinary cortisol excretion, urinary cortisone, tetrahydrocortisol, allo-tetrahydrocortisol, tetrahydrocortison, α-cortolon, and β-cortolon will be measured by using a validated gas chromatography–tandem mass spectrometry and liquid chromatography–tandem mass spectrometry assay (LC-MS/MS) [20,21]. Unbound (free) androgen, cortisol, progesterone, aldosterone, and intermediate metabolites will also be measured using gas chromatography–tandem mass spectrometry as part of a complete urinary steroid profile [20]. Lastly, plasma adrenocorticotropic hormone will be measured. More information on pre-analytical handling can be found in table 2.

Table 2. Sample overview

Sample	Specifications	Centrifuge	Temporary storage on ice?	Tube size	N	Storage temperature
Serum	With gel	1885 g for 5 min on RT	No	500µL	13	−80°C (−112°F)
Serum	Without gel	1300 g for 10 min on 4-8 °C	Yes	1ml/500µL	1/3	−80°C (−112°F)
EDTA plasma		1300 g for 10 min on RT	No	1ml/500µL	1/7	−80°C (−112°F)
EDTA plasma		1300 g for 10 min on 4-8 °C	Yes	1ml/500µL	1/2	−80°C (−112°F)
EDTA plasma*	For pharmacokinetics	1885 g for 5 min on RT	No	1ml/500µL	1/2	−80°C (−112°F)
EDTA	With protease-inhibitors	1100 g for 10 min	No	500µL	2	−80°C (−112°F)
Whole blood**	CYP3A4 and CYP3A5	N.A.	No	4ml	1	−20°C (−4°F)
Sodium fluoride		1300 g for 10 min on 4-8 °C	No	1ml	1	−80°C (−112°F)
Lithium-heparin		1885 g for 5 min on RT	No	500µL	6	−80°C (−112°F)
Lithium-heparin	For PBMC isolation		No	10ml	1	−80°C (−112°F)
PAXgene			No	2.5ml	1	−20°C (−4°F)
24-hour urine		1500 g for 10 min on RT	No	2ml	9	−80°C (−112°F)
Saliva**		N.A.	No	500µL	1	−80°C (−112°F)

N, amount of tubes in storage; PBMC, peripheral blood mononuclear cell; RT, room temperature; *Study visits 1-4; **Only on baseline

Secondary outcomes

Next to the interventional effect on the HPA-axis, the effects on the hypothalamic- pituitary-gonadal axis are studied, taking plasma levels of testosterone, dihydrotestosterone, luteinizing hormone, follicle stimulating hormone, and sex-hormone binding globulin into account. Testosterone and dihydrotestosterone will be measured utilizing LC-MS/MS according to a previously published protocol [22]. To study mineralocorticoid effects, plasma renin and aldosterone, serum potassium, 24h-urine potassium, and transtubular potassium gradient will be determined to assess the effects of prednisolone and dexamethasone on the renin-angiotensin-aldosterone system. The transtubular potassium gradient is used to gauge renal potassium secretion by the cortical collecting duct, providing a good measure of mineralocorticoid bioactivity. First, renin and aldosterone will be measured using an immunoradiometric renin assay (Renin III Generation, Cisbio) and by (LC-MS/MS), respectively, as previously described [23]. Second, both potassium and osmolality (potassium: ion-selective electrode, Roche. Osmolality: method of freezing point depression) will be measured in plasma and in 24-hour urine. These measurements may be taken together utilizing the following formula to calculate the transtubular potassium gradient: $\mathrm{TTPG} = \frac{[K^+]urine}{[K^+]blood} x \frac{Osmblood}{Osmurine}$ [24].

Immune system

To investigate the effect of prednisolone and dexamethasone on the immune system, multiple entities will be investigated. First, absolute leukocyte, granulocyte, and monocyte counts will routinely be performed as outcome and safety measure. Second, during each study visit peripheral blood mononuclear cells will be isolated utilizing Leucosep tubes (227288, Greiner Bio-one, Kremsmünster, Austria). After isolation peripheral blood mononuclear cells will be aliquoted and placed into isopropanol containers and put into nitrogen for long-term storage.

Lastly, to assess the influence of corticosteroids on a gene expression level, 10ml PAXgene tubes will be collected each visit. PAXgene tubes allow for immediate stabilization of intracellular RNA, thereby yielding reproducible and accurate gene expression data.

Pharmacokinetic measurements

Population specific pharmacokinetic models and limited sampling strategy were developed to assess the pharmacokinetic parameters of both prednisolone and dexamethasone (MwPharm version 3.81 (Mediware, Zuidhorn, The Netherlands)). MWPharm parameterized a population pharmacokinetic model, originating from literature values [25]. Population pharmacokinetic models of prednisolone and dexamethasone were described with the following parameters (±SD): bioavailability of 82±13% and 86±5%, absorption constant of 1.6±0.1 h-1 and 0.6±0.0 h-1, volume of distribution of 1.5±0.2L/kg and 2.0±0.5 L/kg, and elimination constant of 0.169±0.033 h-1 and 0.154±0.026 h-1, respectively. Furthermore, Monte Carlo analyses were used to develop the limited sample strategy. In these analyses, 1000 patients were simulated for both dosages of prednisolone and dexamethasone. The area under the curve (AUC) was estimated based on 4 points sampling protocol. Performance criteria were set at a R value of >0.95 and a relative root mean squared error of <15%, table 3 [26].

Table 3. Results of the Monte Carlo analyses for the proposed scheme of four sampling points

	% RMSE
Prednisolone - 7.5 mg	3.34
Prednisolone - 30 mg	2.60
Dexamethasone – 1.125 mg	14.1
Dexamethasone – 4.5 mg	4.66

% RMSE, relative root mean squared error

As a result of these calculations, blood samples will be drawn at three time points, namely before, 3 hours after, and 4 hours after ingestion of the study medication on the 7th day. Furthermore, participants are asked to collect saliva at four time points, with the first three time points corresponding to the blood samples and the fourth 7 to 11 hours after ingestion of the last study medication. Plasma cortisol measurements will be performed using validated LC-MS/MS method [27]. Prednisolone and dexamethasone levels in both plasma and saliva will be measured by isotope dilution LC–MS/MS. Cortisol binding globulin (CBG) will be determined by a radioimmuno-assay, and albumin will be measured using the brome cresol green method on a Roche Modular ISE/P. Individual pharmacokinetic parameters will be calculated by maximum a posteriori Bayesian estimation, essentially performed as described by Werumeus Buning [28]. Total body clearance, volume of distribution, $t_{1/2}$, maximum concentration, and AUC will be calculated for all interventions in each individual. Lastly, CYP3A4 and CYP3A5 polymorphisms will be taken into account, as these genetic variations have an important contribution to inter-individual pharmacokinetic variability.

Anthropometrical and metabolic parameters

Anthropometry measurements will include body length, body weight, waist circumference, and hip circumference. Body weight (kg) will be measured without shoes and outer clothing utilizing a calibrated digital measuring scale (seca 877, seca, Hamburg, Germany). Height (cm) will be measured using a wall-secured stadiometer. Waist and hip circumference (cm) will be calculated using a measuring tape roll with standardized retraction mechanism. Waist circumference will be measured mid-way between the lowest rib and the iliac crest with the participant in standing position. Hip circumference will be determined at the maximum circumference over the trochanter major. All anthropometry measurements will be assessed twice after which the average will be utilized in further analyses.

To assess metabolic function and potential changes during corticosteroid use, we will perform an in-depth analysis of the glucose metabolism and lipid profiles. First, fasting glucose levels will be measured using the Roche P Analyzer and fasting insulin levels and c-peptide levels will be measured utilizing a luminescence-immunoassay (Alinity, Abbot, Abbott Park, Illinois, USA). For glucagon-like peptide-1 (GLP-1) special blood collection tubes will be utilized containing K_2EDTA and a proprietary cocktail which includes esterase inhibitors, dipeptidyl peptidase-4 and other protease-inhibitors (P800 Blood Collection Tube, BD Vacutainer®, Franklin Lakes, NJ, USA). To measure active GLP-1 concentrations, commercially available enzyme-linked immunosorbent assay kit (IBL International (Hamburg, Germany) JP27784) will be utilized.

To further investigate the glucose metabolism, a 75-g oral glucose tolerance test will be performed during all study visits. Venous blood samples will be collected before ingestion and at 30, 60, 90, and 120 minutes after ingestion for measurements of glucose, insulin, C-peptide and GLP-1. All glucose samples will be transported to the clinical laboratory immediately after collection to prevent a decay in the glucose levels due to a delay in preanalytical handling (see table 2) [29].

Furthermore, all samples used to determine lipid levels will be collected after an 8-hour overnight fast. The measurement of total cholesterol, low-density lipoprotein, high density lipoprotein, and triglyceride levels will be performed by our in-hospital routine laboratory. Similarly, for measurement of non-esterified fatty acids, fasting blood samples will be collected and will be analyzed utilizing an enzymatic endpoint method (Diasys kit, Roche, Rotkreuz, Switzerland).

Neurocognitive function

A battery of six standardized cognitive tests, as provided by CanTab Cognitive and Psychological test (CANTAB® (Cognitive assessment software) Cambridge Cognition 2019), covering attention, memory and executive functions will be used. We will use the One Touch Stockings of Cambridge for planning, Paired Associates Learning for visual episodic memory, Rapid Visual Information Processing to test sustained attention, Reaction Time to assess processing and psychomotor speed, and the Motor Screening Task to measure sensorimotor function and comprehension. Practice effects are minimized because this test battery provides parallel modes and stimuli randomization.

Questionnaires

At each study visit participants are asked to complete following questionnaires. The 36-Item Short Form Health Survey (SF-36) is a generic and reliable instrument reflecting 8 domains of health, namely physical functioning, physical role, pain, general health, vitality, social function, emotional role, and mental health [30,31]. The Patient Health Questionnaire-15 (PHQ-15) will be utilized to assess the presences and frequency of adverse events as it is a valuable tool for the detection of somatoform disorders [32]. The Medication Adherence Report Scale (MARS-5) is a short questionnaire measuring participants adherence to the study medication and demonstrates acceptable reliability and validity [33]. The Short Questionnaire to Assess Health-enhancing physical activity (SQUASH) is a valid and reliable questionnaire to assess physical activity levels and contains questions about habitual activities with respect to occupation, leisure time, household, transportation means, and other daily activities [34]. Lastly, as food intake, specifically salt intake, can have an influence on blood pressure and other secondary outcome measures, participants will be asked to complete a 3-day food diary [35].

Biomarkers and other endpoints

Due to the difference in mineralocorticoid effects of prednisolone and dexamethasone, it can be hypothesized that this difference may translate into a difference in blood pressure between prednisolone or dexamethasone treatment. Therefore, blood pressure (mmHg) will be measured according to a standardized clinical protocol using an automated device (Omron M2 Basic, Hoofddorp, The Netherlands). Participants will be seated for at least 15 minutes before blood pressure is measured. Then blood pressure and heart rate are measured three times with a 30 seconds interval.

Hand grip strength will be measured using a Jamar Hydraulic Hand Dynamometer (Patterson Medical JAMAR 5030J1, Warrenville, Canada) as describe previously [36,37]. To measure total body muscle mass, 24h urinary creatinine excretion rate will be utilized as it is an excellent and inexpensive measure of muscle mass [38]. Lastly, osteocalcin will be assessed using electrochemiluminescence immunoassay (Cobas E, Roche, Rotkreuz, Switzerland) as it has been linked to physiological processes such as the glucose metabolism [39].

Assignment of interventions

After enrollment by the study physician (SS or AV), the participant is randomized to start with either prednisolone or dexamethasone in a 1:1 ratio. Randomization will be done by the trial pharmacist of the UMCG in accordance with a pre-specified allocation sequence. Randomization is done using a four-block randomization without stratification. The allocation sequence is stored on a secure network station of the pharmacy of the UMCG.

As the CORE study is designed as a double-blind trial, study participants, study physicians, and principle investigators will be blinded. The blinding is guaranteed by the use of identical study medication capsules and medication labels (Apotheek A15, Gorinchem, The Netherlands). The trial pharmacist who will perform the randomization, will be aware of the

intervention assignment. Unblinding will only be done when a serious adverse event (SAE) occurs, which requires the specific knowledge of the used study medication or when the entire trial is completed. Outcomes will be assessed in a unblinded manor.

Data collection, management, and analysis

Once a participant has given written informed consent, the study will consist of a screening visit and 5 study visits. The latter are a baseline visit, after the low dose of the first intervention, after the high dose of the first intervention, and after the low dose and after the high dose of the second intervention. In principle, all study visits are identical with the exception of the baseline visit where no pharmacological endpoints will be assessed. All data will be collected by two trained study physicians (SPS and AV).

All data, including the questionnaires, will be stored using REDCap (REDCap, Vanderbilt University Medical Center, Nashville, TN, USA). All entered data are double checked by both study physicians. Due to the low risk associated with the study interventions no data monitoring safety board was required. The study will however be intensively monitored, according to the guideline "Quality Assurance of research involving human subjects 2.0" of "The Netherlands Federation of University Medical Centers" [40]. The safety will be assessed in two ways. First, as it is undesirable to use exogenous corticosteroids whilst having an active infection, all participants will be checked for any symptoms (including vital signs, physical examination, and laboratory infection parameters) of an active infection during all study visits. Second, all adverse events, including potential SAE, will be documented and the frequency of all adverse events will therefore be deemed a safety measure.

To ensure confidentiality, all participants will receive a unique identification code, which can only be decoded with a separately stored identification file. As in accordance with

the trial information and consent form, participant information is only accessible to the study physician and study monitor, and in case of a SAE may be provided to the trial pharmacist.

Sample size and statistical analyses

To date, no modern day randomized cross-over trials investigating the effects prednisolone and dexamethasone on the HPA-axis (or other endpoints) in healthy individuals are available. Hence, the number of participants which will be included in the CORE study, is based on the scientific guideline of the European Medicines Agency regarding bio-equivalency studies which states that bio-equivalence studies should not include less than 12 subjects [41]. Because males and females clinically differ in terms of circulating levels of oestrogens and corresponding CBG levels, we deemed it necessary to included 12 male and 12 female participants. If drop-out cannot be prevented, new volunteers will be included to ensure adherence to the scientific guideline of the European Medicines Agency.

Descriptive statistics will be utilized to characterize the study population and for all study endpoints Wilcoxon Signed Ranks Test will be utilized. As the anticipated duration of the trial is expected to be limited no interim analyses will be performed. The newest versions of IBM Statistics SPSS (IBM Inc. Chicago, IL, USA), GraphPad Prism (La Jolla, CA, USA), STATA (STATA Corp., TX, USA), and/or R (Vienna, Austria) will be used for statistical analyses.

Dissemination

This study will be submitted for publication in peer reviewed journals and oral presentations at (inter)national conferences. Authorships will be determined based on the International Committee of Medical Journal Editors guidelines. Raw data will be available upon reasonable request in de-identified form.

Trial status

The CORE study has started on the 4th of March 2021. On 1st of January 2022 fifteen participants have concluded the study. A total inclusion time of one year and three months was anticipated, however due to the COVID-19 pandemic the complementing vaccination campaign, the study inclusion is delayed. The extent of the delay is at this moment still unclear.

Discussion

This article describes the rationale and design of the CORE study which is a randomized, double-blind, cross-over trial investigating the bio-equivalence and dose response of prednisolone and dexamethasone with regard to various physiological systems of the human body. Within this design, the CORE study will include 12 healthy men and 12 healthy women, to receive 7.5 mg prednisolone/1.125 mg dexamethasone and 30 mg prednisolone/4.5 mg dexamethasone all for one week in random order. Data will be collected to evaluate hormonal axes, immunological status, metabolic pathways, pharmacokinetic parameters, and other organ systems with state-of-the-art laboratory techniques.

Although prednisolone and dexamethasone are already widely used in clinical practice, well-validated bio-equivalence data are lacking. The CORE study will help to gain new insight into the comparability between the two medications and improve the existing pharmacodynamic data. By investigating outcome measurements in a cross-over and double-blind fashion, in-depth information regarding the system specific effects of prednisolone and dexamethasone will be gained whilst taking inter-individual differences into account. Another strength of the CORE study the selected dosage and treatment duration reflect clinical practice. This will aid translating the outcomes of the CORE study to routine clinical practice. A limitation of the current study is the absence of a placebo arm. Inclusion of a placebo intervention, however, may result in a substantial increase of the study duration, and may subsequently result in negative effect on the inclusion rate. As a result, a baseline study visit was implemented to serve as reference point. Another limitation could be the relative low number of participants. Nevertheless, the number of included participants is in concordance with current guidelines of bio-equivalence study of the European Medicines Agency and is even double the number of minimal requirement of subjects, to allow for subgroup analyses based on age and sex.

In conclusion, the CORE study has the potential to improve the current understanding of the most widely used corticosteroids and may therefore aid various clinicians in clinical decision making, including general practitioners, endocrinologists, nephrologists, rheumatologists and many more.

Declarations

Acknowledgements

We would like to acknowledge the help of Tanja Zijp, PharmD with all pharmacokinetics timepoint calculations. In addition, we would like to extent our gratitude to Elisabeth Raveling-Eelsing for performing all peripheral blood mononuclear cell isolations.

Author contributions

SJB and APvB are the principal investigators and devised the original draft of the study design. MJV and IPK have provided valuable input on the utilized laboratory measurement and methods. MNK has aided in critical review of the current manuscript. AR has facilitated the collection of the immunological study endpoints and provided vital knowledge to the design of the study. DJT has provided crucial input with regard to the pharmacological aspects of the interventions and the study design. SPS and AV are collecting the data and wrote the first draft of the design paper. All authors have read and approved the manuscript.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Institutional Review Board Statement

The CORE study has been approved by the Medical Ethics Committee of the UMCG (2020.398) on 18th of January 2021. The trial was designed and implemented in accordance with the Good Clinical Practice guidelines and follows the International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human

Use (including European Directive 2001/20/EC) and adherence to the ethical principles as laid down in the Declaration of Helsinki, Brazil, October 2013.

Informed Consent Statement

All participants will have to provide written informed consent prior to the enrollment in the trial. The informed consent contains a subsection on the storage and future use of any bodily materials collected during the trial.

Conflict of interest

The authors declare no conflict of interest. Furthermore, the sponsors had no role in the design, execution, interpretation, or writing of the study.

References

1 Benedek TG. History of the development of corticosteroid therapy. *Clin Exp Rheumatol* 2011;**29**.

2 Fardet L, Petersen I, Nazareth I. Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years. *Rheumatology (Oxford)* 2011;**50**:1982–90.

3 Furst DE, Saag KG. Determinants of glucocorticoid dosing. UpToDate, Post TW (Ed), UpToDate, Waltham, MA. https://www.uptodate.com/contents/determinants-of-glucocorticoid-dosing?sectionName=BIOEQUIVALENCE AND BIOAVAILABILITY&topicRef=124&anchor=H2&source=see_link#H2 (accessed 9 Dec 2021).

4 Herzog H, Nobile A, Tolksdorf S, *et al.* New Antiarthritic Steroids. *Science.* 1955;**121**.

5 Boland EW. 16a-Methyl corticosteroids; a new series of anti-inflammatory compounds; clinical appraisal of their antirheumatic potencies. *Calif Med* 1958;**88**:417–22.

6 Krasowski MD, Drees D, Morris CS, *et al.* Cross-reactivity of steroid hormone immunoassays: Clinical significance and two-dimensional molecular similarity prediction. *BMC Clin Pathol* 2014;**14**:1–13.

7 Peters WP, Holland JF, Senn H, *et al.* Corticosteroid administration and localized leukocyte mobilization in man. *N Engl J Med* 1972;**286**:342–5.

8 Meikle AW, Tyler FH. Potency and duration of action of glucocorticoids. Effects of hydrocortisone, prednisone and dexamethasone on human pituitary-adrenal function. *Am J Med* 1977;**63**:200–7.

9 Melby JC. Systemic corticosteroid therapy: pharmacology and endocrinologic considerations. *Ann Intern Med* 1974;**81**:505–12.

10 Mager DE, Lin SX, Blum RA, *et al.* Dose Equivalency Evaluation of Major Corticosteroids: Pharmacokinetics and Cell Trafficking and Cortisol Dynamics. *J Clin Pharmacol* 2003;**43**:1216–27.

11 Schimmer BP, Funder JW. ACTH, Adrenal Steroids, and Pharmacology of the Adrenal Cortex. In: Brunton LL, Chabner BA, Knollmann BC, eds. *Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 12e*. New York, NY: : McGraw-Hill Education 2015.

12 Diederich S, Eigendorff E, Burkhardt P, *et al.* 11β-hydroxysteroid dehydrogenase types 1 and 2: An important pharmacokinetic determinant for the activity of synthetic mineralo- and glucocorticoids. *J Clin Endocrinol Metab* 2002;**87**:5695–701.

13 Nicolaides NC, Charmandari E, Chrousos GP, *et al.* Recent advances in the molecular mechanisms determining tissue sensitivity to glucocorticoids: Novel mutations,

circadian rhythm and ligand-induced repression of the human glucocorticoid receptor. *BMC Endocr Disord* 2014;**14**:1–12.

14 Kino T. Tissue Glucocorticoid Sensitivity: Beyond Stochastic Regulation on the Diverse Actions of Glucocorticoids. *Horm Metab Res* 2007;**39**:420–4.

15 Czock D, Keller F, Rasche FM, *et al.* Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clin Pharmacokinet* 2005;**44**:61–98.

16 Werumeus Buning J, Touw DJ, Brummelman P, *et al.* Pharmacokinetics of oral hydrocortisone - Results and implications from a randomized controlled trial. *Metabolism* 2017;**71**:7–16.

17 Vulto A, Minović I, de Vries L V., *et al.* Endogenous urinary glucocorticoid metabolites and mortality in prednisolone-treated renal transplant recipients. *Clin Transplant* 2020;**34**:1–14.

18 Furst DE, Saag KG. Glucocorticoid withdrawal. UpToDate, Post TW (Ed), UpToDate, Waltham, MA. (Accessed 9 Dec 2021)

19 Prete A, Bancos I. Glucocorticoid induced adrenal insufficiency. *Br Med J* 2021;**374**:n.1380.

20 Jong WHAD, Buitenwerf E, Pranger AT, *et al.* Determination of reference intervals for urinary steroid profiling using a newly validated GC-MS/MS method. *Clin Chem Lab Med* 2017;**56**:103–12.

21 de Vries L V., de Jong WHA, Touw DJ, *et al.* Twenty-four hour urinary cortisol excretion and the metabolic syndrome in prednisolone-treated renal transplant recipients. *Steroids* 2017;**127**:31–9.

22 van der Veen A, van Faassen M, de Jong WHA, *et al.* Development and validation of a LC-MS/MS method for the establishment of reference intervals and biological variation for five plasma steroid hormones. *Clin Biochem* 2019;**68**:15–23.

23 Van Der Gugten JG, Crawford M, Grant RP, *et al.* Supported liquid extraction offers improved sample preparation for aldosterone analysis by liquid chromatography tandem mass spectrometry. *J Clin Pathol* 2012;**65**:1045–8.

24 West ML, Bendz O, Chen CB, *et al.* Development of a test to evaluate the transtubular potassium concentration gradient in the cortical collecting duct in vivo. *Miner Electrolyte Metab* 1986;**12**:226–33.

25 Prednisolone and Dexamethasone. In: DrugDex [database on the Internet]. Greenwood Village (CO): IBM Corporation; 2020 [cited 09-11-2020]. Available from: www.micromedexsolutions.com. Subscription required to view.

26 Van den Elsen SHJ, Sturkenboom MGG, Van't Boveneind-Vrubleuskaya N, *et al.* Population pharmacokinetic model and limited sampling strategies for personalized dosing of levofloxacin in tuberculosis patients. *Antimicrob Agents Chemother* 2018;**62**.

27 van Faassen M, Bischoff R, Kema I. Relationship between plasma and salivary melatonin and cortisol investigated by LC-MS/MS. *Clin Chem Lab Med* 2017;**55**:1340–948.

28 Werumeus Buning J, Touw DJ, Brummelman P, *et al.* Pharmacokinetics of oral hydrocortisone - Results and implications from a randomized controlled trial. *Metabolism* 2017;**71**:7–16.

29 Potter JM, Hickman PE, Oakman C, *et al.* Strict preanalytical oral glucose tolerance test blood sample handling is essential for diagnosing gestational diabetes mellitus. *Diabetes Care* 2020;**43**:1438–41.

30 Brazier JE, Harper R, Jones NMB, *et al.* Validating the SF-36 health survey questionnaire: New outcome measure for primary care. *Br Med J* 1992;**305**:160–4.

31 Cech DJ, Martin S "Tink". Chapter 5 - Evaluation of Function, Activity, and Participation. In: Cech DJ, Martin S "Tink", eds. *Functional Movement Development Across the Life Span (Third Edition)*. Saint Louis: : W.B. Saunders 2012. 88–104.

32 Kroenke K, Spitzer RL, Williams JBW. The PHQ-15: Validity of a new measure for evaluating the severity of somatic symptoms. *Psychosom Med* 2002;**64**:258–66.

33 Chan AHY, Horne R, Hankins M, *et al.* The Medication Adherence Report Scale: A measurement tool for eliciting patients' reports of nonadherence. *Br J Clin Pharmacol* 2020;**86**:1281–8.

34 Wendel-Vos GCW, Schuit AJ, Saris WHM, *et al.* Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *J Clin Epidemiol* 2003;**56**:1163–9.

35 Yang YJ, Kim MK, Hwang SH, *et al.* Relative validities of 3-day food records and the food frequency questionnaire. *Nutr Res Pract* 2010;**4**:142.

36 Angst F, Drerup S, Werle S, *et al.* Prediction of grip and key pinch strength in 978 healthy subjects. *BMC Musculoskelet Disord* 2010;**11**:2–7.

37 Eisenga MF, Gomes-Neto AW, Van Londen M, *et al.* Rationale and design of TransplantLines: A prospective cohort study and biobank of solid organ transplant recipients. *BMJ Open* 2018;**8**:1–13.

38 Heymsfield SB, Arteaga C, McManus C, *et al.* Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr* 1983;**37**:478–94.

39 Moser SC, van der Eerden BCJ. Osteocalcin — A versatile bone-derived hormone. *Front Endocrinol (Lausanne)* 2019;**10**:4–9.

40 Guideline Quality assurance of research involving human subjects. https://www.nfu.nl/sites/default/files/2021-

01/21.00024_Guideline_Quality_assurance_of_research_involving_human_subjects_dec20_0.pdf. (accessed 30 Mar 2021).

41 European Medicines Agency. GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE. 2010. Doc. Ref.: CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **

Figure legend

Figure 1. IC, Informed consent; B, baseline; RND, randomization; WO, wash-out; w, weeks

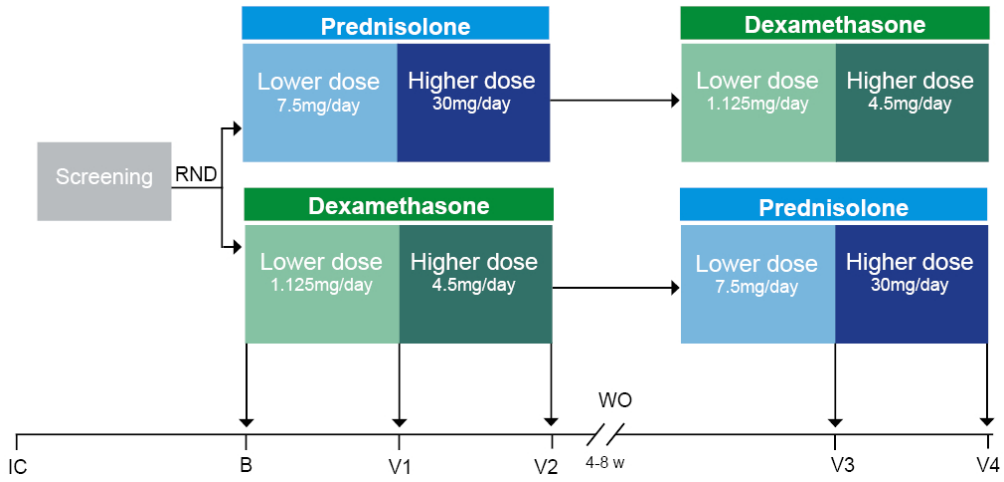

Figure 1. IC, Informed consent; B, baseline; RND, randomization; WO, wash-out; w, weeks

672x320mm (38 x 38 DPI)

Supplemental Material

Rationale and Design of the CORE (COrticosteroids REvised) study: A Randomized Cross-over Clinical Trial of Prednisolone versus Dexamethasone

Suzanne P. Stam, MD[1]*, Annet Vulto, MD[2]*, Michel J. Vos, PhD[3], Michiel N. Kerstens, MD,PhD[2], Abraham Rutgers, MD, PhD[4], Ido P. Kema, PhD[3], Daan J. Touw, PharmD, PhD[5], Stephan J.L. Bakker MD,PhD[1], André P. van Beek, MD,PhD[2]

* contributed equally

[1] Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[2] Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[3] Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands

[4] Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[5] Department of Clinical Pharmacy and Pharmacology, University of Groningen, Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands

Corresponding author:

A.P. van Beek, MD PhD

Department of Internal Medicine, Division of Endocrinology

University Medical Center Groningen

P.O. Box 30.001, 9700 RB Groningen, the Netherlands

Phone: 0031 050 361 0388, Email: a.p.van.beek@umcg.nl

Appendix A

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	**1**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	**2, 5, 11**
	2b	All items from the World Health Organization Trial Registration Data Set	**N.A.**
Protocol version	3	Date and version identifier	**2**
Funding	4	Sources and types of financial, material, and other support	**2, 26**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	**1-3, 26**
	5b	Name and contact information for the trial sponsor	**3**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	**N.A.**
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	**N.A.**
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	**7-8**
	6b	Explanation for choice of comparators	**7**
Objectives	7	Specific objectives or hypotheses	**8**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	**9**

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	**9**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	**9,10,21**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	**12**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	**21**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	**12,19**
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	**10**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	**12-20**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	**9, 21, See fig 1**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	**22**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	**9,11**
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	**20**

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	**20**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	**20**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	**20,21**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	**21**
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	**12-20**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	**22**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	**21,22**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	**22**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	**N.A.**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	**22**
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	**21**

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	**N.A.**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	**19,22**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	**21**
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	**11**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	**11**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	**11**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	**11**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	**21,22**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	**27**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	**N.A.**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	**11**
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	**22**
	31b	Authorship eligibility guidelines and any intended use of professional writers	**22**
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	**22**

Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	**App B**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	**14**

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Appendix B

Toestemmingsformulier proefpersoon

Een nieuwe blik op corticosteroïden

- Ik heb de informatiebrief gelezen. Ook kon ik vragen stellen. Mijn vragen zijn voldoende beantwoord. Ik had genoeg tijd om te beslissen of ik meedoe.
- Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen of te stoppen met het onderzoek. Daarvoor hoef ik geen reden te geven.
- Ik geef toestemming voor het informeren van mijn huisarts dat ik meedoe aan dit onderzoek
- Ik geef toestemming voor het verzamelen en gebruiken van mijn gegevens, bloedmonsters en lichaamsmateriaal voor de beantwoording van de onderzoeksvraag in dit onderzoek
- Ik weet dat voor de controle van het onderzoek sommige mensen toegang tot al mijn gegevens kunnen krijgen. Die mensen staan vermeld in deze informatiebrief. Ik geef toestemming voor die inzage door deze personen.
- Ik geef toestemming voor het informeren van mijn huisarts en/of behandelend specialist van onverwachte bevindingen die van belang (kunnen) zijn voor mijn gezondheid.
- Ik weet dat ik niet zwanger mag worden tijdens het onderzoek.
- Vrouwelijke deelneemsters: de onderzoeker heeft het gebruik van anticonceptie met mij besproken.
- Ik geef □ **wel**

  □ **geen**

  toestemming om mijn persoonsgegevens langer te bewaren en te gebruiken voor toekomstig onderzoek op het gebied van de onderzoeksmedicatie.
- Ik geef □ **wel**

  □ **geen**

  toestemming om mijn lichaamsmateriaal na dit onderzoek te bewaren en om dit later nog voor meer onderzoek te gebruiken, zoals in de informatiebrief staat.
- Ik geef □ **wel**

  □ **geen**

  toestemming om mij na dit onderzoek opnieuw te benaderen voor een vervolgonderzoek.
- Ik wil □ **wel**

  □ **niet**

  geïnformeerd worden over welke behandeling ik heb gehad/in welke groep ik zat.

- **Ik wil meedoen aan dit onderzoek.**

Naam proefpersoon:

Handtekening: Datum : __ / __ / __

Ik verklaar dat ik deze proefpersoon volledig heb geïnformeerd over het genoemde onderzoek.

Als er tijdens het onderzoek informatie bekend wordt die de toestemming van de proefpersoon zou kunnen beïnvloeden, dan breng ik hem/haar daarvan tijdig op de hoogte.

Naam onderzoeker (of diens vertegenwoordiger):

Handtekening: Datum: __ / __ / __

---

* Doorhalen wat niet van toepassing is.

De proefpersoon krijgt een volledige informatiebrief mee, samen met een getekende versie van het toestemmingsformulier.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	**1**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	**2, 5, 11**
	2b	All items from the World Health Organization Trial Registration Data Set	**N.A.**
Protocol version	3	Date and version identifier	**2**
Funding	4	Sources and types of financial, material, and other support	**2, 26**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	**1-3, 26**
	5b	Name and contact information for the trial sponsor	**3**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	**N.A.**
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	**N.A.**
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	**7-8**
	6b	Explanation for choice of comparators	**7**
Objectives	7	Specific objectives or hypotheses	**8**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	**9**

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	**9**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	**9,10,21**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	**12**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	**21**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	**12,19**
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	**10**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	**12-20**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	**9, 21, See fig 1**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	**22**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	**9,11**
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	**20**

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	**20**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	**20**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	**20,21**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	**21**
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	**12-20**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	**22**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	**21,22**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	**22**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	**N.A.**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	**22**
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	**21**

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	**N.A.**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	**19,22**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	**21**
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	**11**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	**11**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	**11**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	**11**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	**21,22**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	**27**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	**N.A.**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	**11**
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	**22**
	31b	Authorship eligibility guidelines and any intended use of professional writers	**22**
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	**22**

Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	**App B**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	**14**

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Rationale and Design of the CORE (COrticosteroids REvised) study: Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-061678.R1
Article Type:	Protocol
Date Submitted by the Author:	07-Apr-2022
Complete List of Authors:	Stam, Suzanne; University Medical Centre Groningen, Internal Medicine, division of Nephrology Vulto, Annet; University Medical Centre Groningen, Internal Medicine, division of Endocrinology Vos, Michel; University Medical Centre Groningen, Laboratory Medicine Kerstens, Michiel; University Medical Centre Groningen, Internal Medicine, division of Endocrinology Rutgers, Abraham; University Medical Centre Groningen, Rheumatology and Clinical Immunology Kema, Ido; University Medical Centre Groningen, Laboratory Medicine Touw, Daan; University Medical Centre Groningen, Clincal Pharmacy and Pharmacology Bakker, Stephan; University Medical Centre Groningen, Internal Medicine, division of Nephrology van Beek, André; University Medical Centre Groningen, Internal Medicine, division of Endocrinology
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Evidence based practice, Immunology (including allergy), Pharmacology and therapeutics
Keywords:	General endocrinology < DIABETES & ENDOCRINOLOGY, Sex steroids & HRT < DIABETES & ENDOCRINOLOGY, CLINICAL PHARMACOLOGY, INTERNAL MEDICINE

Rationale and Design of the CORE (COrticosteroids REvised) study: Protocol

Suzanne P. Stam, MD[1]*, Annet Vulto, MD[2]*, Michel J. Vos, PhD[3], Michiel N. Kerstens, MD,PhD[2], Abraham Rutgers, MD, PhD[4], Ido P. Kema, PhD[3], Daan J. Touw, PharmD, PhD[5], Stephan J.L. Bakker MD,PhD[1], André P. van Beek, MD,PhD[2]

* contributed equally

[1] Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[2] Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[3] Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands

[4] Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[5] Department of Clinical Pharmacy and Pharmacology, University of Groningen, Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands

Running title:

Randomized clinical trial on Prednisolone and Dexamethasone Bio-equivalency

Names for PubMed Indexing:

Stam SP, Vulto A, Vos MJ, Kerstens MN, Rutgers A, Kema IP, Touw DJ, Bakker SJL, Van Beek AP

Word count: 4103. Abstract words: 244

Tables: 3 Figure: 1

Corresponding author:

A.P. van Beek, MD PhD

Department of Internal Medicine, Division of Endocrinology

University Medical Center Groningen

P.O. Box 30.001, 9700 RB Groningen, the Netherlands

Phone: 0031 050 361 0388, Email: a.p.van.beek@umcg.nl

Administrative information

Title {1}	Rationale and Design of the CORE (COrticosteroids REvised) study: A Protocol
Trial registration {2a and 2b}.	EudraCT: 2019-004983-23 ClinicalTrial.gov: NCT04733144 Dutch trial registry (NTR): NL9138
Protocol version {3}	3.0; date 23rd December 2020
Funding {4}	Investigator-initiated
Author details {5a}	Suzanne P. Stam: Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. Annet Vulto: Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. Michel J. Vos: Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands Michiel N. Kerstens: Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. Abraham Rutgers: Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. Ido P. Kema: Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

	Daan J. Touw: Department of Clinical Pharmacy and Pharmacology, University of Groningen, Groningen, University Medical Center Groningen, Groningen, The Netherlands Stephan J.L. Bakker: Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands. André P. van Beek: Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.
Name and contact information for the trial sponsor {5b}	University Medical Center Groningen Hanzeplein 1, Postbus 30001, 9700 RB Groningen, The Netherlands
Role of sponsor {5c}	N.A.

Abbreviations

AUC	-	Area under the curve
CBG	-	Cortisol binding globulin
GLP-1	-	Glucagon-like peptide-1
GR	-	Glucocorticoid receptor
HPA-axis	-	Hypothalamic pituitary adrenal axis
LC-MS/MS	-	Liquid chromatography–tandem mass spectrometry
MR	-	Mineralocorticoid receptor
SAE	-	Serious adverse event
UMCG	-	University Medical Center Groningen

Abstract

Introduction. Corticosteroids are an important pillar in many anti-inflammatory and immunosuppressive treatment regimens and are available in natural and synthetic forms, which are considered equipotent if clinical bio-equivalence data are utilized. Current clinical bio-equivalence data are however based on animal studies or studies with subjective endpoints. Furthermore, advancement in steroid physiology with regard to metabolism, intracellular handling and receptor activation have not yet been incorporated. Therefore, this study aims to re-examine the clinical bio-equivalence and dose effects of the most widely used synthetic corticosteroids, prednisolone and dexamethasone.

Methods and analysis. In this double-blind, randomized cross-over clinical trial, 24 healthy male and female volunteers aged 18-75 years, will be included. All volunteers will randomly receive either first a daily dose of 7.5 mg prednisolone for one week, immediately followed by a daily dose of 30 mg prednisolone for one week, or first a presumed clinical bio-equivalent dose of 1.125 mg dexamethasone per day, immediately followed by 4.5 mg of dexamethasone per day for one week. After a 4–8 week wash-out period the other treatment will be applied. The primary study endpoint is the difference in free cortisol excretion in 24h urine. Secondary endpoints will include differences in immunological parameters, blood pressure, and metabolic measurements.

Ethics and dissemination. This study has been approved by the Medical Ethics Committee of the University Medical Center Groningen (METC 2020.398) and registered on ClinicalTrials.gov (NCT04733144). The results of this study will be submitted for publication in peer reviewed journals.

Keywords

Corticosteroids, Prednisolone, Dexamethasone, bio-equivalence, healthy subjects

Strengths and Limitations

1. Cross-over design limits high inter-individual effect of exogenous glucocorticoids
2. State-of-the-art laboratory techniques, consisting of validated gas chromatography–tandem mass spectrometry and liquid chromatography–tandem mass spectrometry assays, which have superior specificity compared to immunoassays
3. Utilized doses reflect clinical practice
4. Absence of placebo intervention
5. Due to the COVID-19 pandemic and subsequent vaccination campaign wash-out could not always be maintained at 4 to 8 weeks.

Introduction

Since the first clinical use of cortisone in 1948, glucocorticoids have become a fundamental part in the treatment of many diseases, including autoimmune disorders, respiratory disorders, and haematological malignancies [1]. Furthermore, corticosteroids have become a mainstay in the immunosuppressive treatment for solid organ transplantation. Corticosteroids are available in various natural and synthetic forms [2]. In a clinical setting, different natural and synthetic forms are applied interchangeably, for which equipotent doses can be calculated according to established clinical bio-equivalence data [3]. Although this is more or less thoughtlessly applied in daily practice, it is important to realise that the literature which provides the rationale for the current clinical bio-equivalence data, consists of old, non-randomized studies [4,5]. In addition, these studies are limited by the use of subjective endpoints, outdated laboratory techniques, and the use of animals or patients with rheumatoid arthritis as study participants [4–6]. Later, some attempts have been made to improve clinical bio-equivalence data of corticosteroids, but these attempts were hampered by methodological imperfections [7–9]. Since then one pharmacological study, performed approximately twenty years ago, suggested that the current dosing tables reflect a reasonable dose equivalence relation, but this study included only 5 men and described only the effects of a single interventional dose [10]. Furthermore, recent decades have resulted in major advancements in our knowledge of corticosteroids, especially on intracellular handling and receptor transactivation or –repression but this has not yet resulted in a better understanding of their clinical bioequivalence.

Predniso(lo)ne and dexamethasone are the most commonly prescribed representatives of the synthetic corticosteroids and therefore provide an important focus to study clinical bio-equivalence. When studying this, effects on the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), metabolism or intracellular handling as well as tissue or system specific transactivation or -repression should be taken into account. Regarding the first,

predniso(lo)ne and dexamethasone have divergent effects, because whilst both have GR effects (i.e. anti-inflammatory and immunosuppressive properties), only predniso(lo)ne has MR effects [11]. Although these characteristics are known since their discovery, it may have important consequences for various organ systems relying on mineralocorticoid effects such as the brain and kidney, resulting in different (side) effects. Novel insights have also unveiled a difference in metabolism, for example due to an alternative intracellular handling by both 11β-hydroxysteroid dehydrogenase type 1 and type 2 [12]. It can therefore be hypothesized that currently presumed equipotent doses of prednisolone and dexamethasone have different effects on various organ systems for which these enzymes are important. Also, advancement in the understanding of the molecular mechanism of the GR has uncovered a wide range of system specific sensitivities to corticosteroids [13,14]. This indicates that the currently used approach of one conversion factor for all body systems may not be justified. Instead, it may be necessary to take this heterogenicity into account, by utilizing system specific conversion rates. Finally, as studies have demonstrated that the pharmacokinetics of prednisolone are non-linear, whilst those of dexamethasone are, it may be postulated that the conversion factor between prednisolone and dexamethasone is dose-dependent [15].

Therefore, we aim to re-examine the clinical bio-equivalence and dosing effects of prednisolone and dexamethasone on various physiological systems, to provide reliable in vivo data in healthy volunteers and thus provide data to optimize systemic corticosteroid therapy to modern day standards.

Methods and analysis

Study design

The CORE study is an investigator-initiated, single-center, randomized, double-blind, crossover trial including healthy volunteers to receive two doses of prednisolone and two doses of dexamethasone. All volunteers will be randomly assigned to receive either first a daily dose of 7.5 mg prednisolone for one week, immediately followed by a daily dose of 30 mg of prednisolone for one week, or first a presumed clinical bio-equivalent dose of 1.125 mg dexamethasone per day, immediately followed by 4.5 mg of dexamethasone per day for one week (figure 1). After a four to eight week wash-out period the other treatment will be applied. The duration of the wash-out period is at least four weeks, but can be extended to eight weeks to prevent the influence of stressful periods such as exams and work deadlines. The primary outcomes of the trial is the difference in 24h urinary free cortisol excretion between lowest doses and highest doses of prednisolone and dexamethasone.

Study setting and population

All study visits will be performed in the outpatient clinic of the University Medical Center Groningen (UMCG), an academic hospital in the northern part of the Netherlands. A total of 24 healthy volunteers will be included in the study. As most of the outcomes are dependent on age and sex, the participants are subdivided into 4 groups, specifically 6 males aged 18-50 years, 6 females aged 18-50 years and using oral contraceptives, 6 males aged ≥50-75 years, and lastly 6 postmenopausal females aged ≥50-75 years. Next to the age and hormonal status mentioned above, volunteers need to have a BMI between 18.5-30 kg/m^2, no relevant medical history, and no dependency on any type of corticosteroid in any pharmaceutical form. All inclusion and exclusion criteria can be found in table 1. Participants will either be recruited through pamphlets placed in local public buildings or advertisement in the local newspaper.

Table 1. Inclusion and exclusion criteria for the CORE study

Inclusion criteria	Exclusion criteria
1. Participants must have good command of the Dutch language 2. Participants must provide written informed consent 3. Participants must have an age between 18 – 75 years old 4. Female participants aged 18-49 years must be using oral contraceptives and female participants age 50-75 years must be in the postmenopausal state 5. BMI between 18.5 and 30 kg/m2 6. Participants are not allowed to have a relevant medical history or use interfering medication	1. Potential participants with a medical history of: a. Diseases affecting the HPA-axis: e.g. primary and secondary adrenal insufficiency, pituitary tumors, or Cushings' disease b Chronic inflammatory diseases: e.g. rheumatoid arthritis, polymyalgia rheumatica, and asthma c. Psychiatric diseases d. Diabetes mellitus 2. Potential participants who have known contraindication to the study medication (e.g. known peptic ulcer disease or active infectious disease) 3. Night shift workers 4. Potential participants with a kidney function <60 ml/min/1.73m2, abnormalities in liver enzymes, and/or abnormalities in thyroid function 5. Potential participants who are dependent on corticosteroids in any form, e.g. asthmatic patients, and transplant recipients 6. Potential participants who utilize any medication which is likely to confound assessment of one the endpoints (e.g. inhaled corticosteroids, hormone supplements, psychotropic drugs, carbamazepine or vaccination) 7. Potential participants who intend to undergo significant lifestyle changes e.g. voluntary weight loss and discontinue smoking habits. 8. Potential participants who are unlikely to adhere to the study medication (e.g. volunteers with a history of substance abuse or non-adherence)

Patient and Public Involvement

As this study is performed with healthy subjects, patients were not directly involved to the design of the study. Recruitment of participants was however updated based on input of the volunteers.

Intervention

This study is designed as a crossover trial as previous studies have demonstrated a high inter-individual variation for the effect of exogenous corticosteroids [16,17]. One intervention consists of two doses of prednisolone (11β,17,21-trihydroxy-1,4-pregnadieen-3,20-dion). To align the CORE study as much as possible with current clinical practice, the doses that were chosen were based on dosages which are often prescribed in clinical practice. In general, a distinction is made between maintenance doses, ranging from 5-20 mg prednisolone daily and active treatment doses, ranging from 30-80 mg prednisolone daily. To minimize potential side effects, we selected a low maintenance dose at a borderline physiological level, namely 7.5 mg prednisolone and a low active treatment dose namely 30 mg prednisolone, both for the duration of a week. To allow for comparison between prednisolone and dexamethasone, the currently presumed clinical bio-equivalency data of dexamethasone (9-fluor-11β,17,21-trihydroxy-16α-methyl-1,4-pregnadieen-3,20-dion) were used, resulting in 1.125 mg dexamethasone and 4.5 mg dexamethasone, respectively [18]. All study medication was taken every day at eight o'clock in the morning after an overnight fast and provided to participants as capsules for oral ingestion. No tapering is applied as both intervention periods are no longer than two weeks [19]. To monitor interventional adherence, all remain drug capsules were counted upon return during the study visit.

Primary outcome

24h urinary cortisol excretion

The primary composite endpoint is the difference between the two lower doses and two higher doses of prednisolone and dexamethasone measured by 24h urinary free cortisol excretion as measure for hypothalamic-pituitary-adrenal axis (HPA-axis) suppression (24h free cortisol $Pred_{7.5mg}$ – $Dex_{1.125mg}$ and 24h free cortisol $Pred_{30mg}$ – $Dex_{4.5mg}$). For this endpoint, 24h urine is collected according to a strict protocol which is as follows: on the morning of the day before a study visit, participants are asked to discard a urine void and subsequently collect all urine for the next 24h including a urine void at exactly 24h after the first discarded urine void. Next to 24h urinary free cortisol excretion, urinary cortisone, tetrahydrocortisol, allo-tetrahydrocortisol, tetrahydrocortison, α-cortolon, and β-cortolon will be measured by using a validated gas chromatography–tandem mass spectrometry and liquid chromatography–tandem mass spectrometry assay (LC-MS/MS) [20,21].

Androsterone, etiocholanolone, dehydroepiandrosterone, 11-Keto-etiocholanolone, 11-Hydroxyandrosterone, 11-Hydroxyetiocholanolone and estriol will also be measured using gas chromatography–tandem mass spectrometry as part of a complete urinary steroid profile, as well as allo-pregnanediol, pregnanediol, pregnanetriol and polone. Furthermore, 11-dehydrotetrahydrocorticosterone, tetrahydrocorticosterone, allo-tetrahydrocorticosterone, tetrahydrodeoxycortisol, pregnanediolone, pregnanetriolone, allo-pregnanediolone and 11-deoxytetrahydrocorticosterone will be measured in the same GC-MS/MS assay [20]. Additionally, plasma adrenocorticotropic hormone will be measured. More information on pre-analytical handling can be found in table 2.

Table 2. Sample overview

Sample	Specifications	Centrifuge	Temporary storage on ice?	Tube size	N	Storage temperature
Serum	With gel	1885 g for 5 min on RT	No	500µL	13	−80°C (−112°F)
Serum	Without gel	1300 g for 10 min on 4-8 °C	Yes	1ml/500µL	1/3	−80°C (−112°F)
EDTA plasma		1300 g for 10 min on RT	No	1ml/500µL	1/7	−80°C (−112°F)
EDTA plasma		1300 g for 10 min on 4-8 °C	Yes	1ml/500µL	1/2	−80°C (−112°F)
EDTA plasma*	For pharmacokinetics	1885 g for 5 min on RT	No	1ml/500µL	1/2	−80°C (−112°F)
EDTA	With protease-inhibitors	1100 g for 10 min	No	500µL	2	−80°C (−112°F)
Whole blood**	CYP3A4 and CYP3A5	N.A.	No	4ml	1	−20°C (−4°F)
Sodium fluoride		1300 g for 10 min on 4-8 °C	No	1ml	1	−80°C (−112°F)
Lithium-heparin		1885 g for 5 min on RT	No	500µL	6	−80°C (−112°F)
Lithium-heparin	For PBMC isolation		No	10ml	1	−80°C (−112°F)
PAXgene			No	2.5ml	1	−20°C (−4°F)
24-hour urine		1500 g for 10 min on RT	No	2ml	9	−80°C (−112°F)
Saliva**		N.A.	No	500µL	1	−80°C (−112°F)

N, amount of tubes in storage; PBMC, peripheral blood mononuclear cell; RT, room temperature; *Study visits 1-4; **Only on baseline

Secondary outcomes

Next to the interventional effect on the HPA-axis, the effects on the hypothalamic- pituitary-gonadal axis are studied, taking plasma levels of testosterone, dihydrotestosterone, progesterone, 17-hydroxyprogesterone, androstenedione, luteinizing hormone, follicle stimulating hormone, and sex-hormone binding globulin into account. Testosterone and dihydrotestosterone will be measured utilizing LC-MS/MS according to a previously published protocol [22]. To study mineralocorticoid effects, plasma renin and aldosterone, serum potassium, 24h-urine potassium, and transtubular potassium gradient will be determined to assess the effects of prednisolone and dexamethasone on the renin-angiotensin-aldosterone system. The transtubular potassium gradient is used to gauge renal potassium secretion by the cortical collecting duct, providing a good measure of mineralocorticoid bioactivity. First, renin and aldosterone will be measured using an immunoradiometric renin assay (Renin III Generation, Cisbio) and by (LC-MS/MS), respectively, as previously described [23]. Second, both potassium and osmolality (potassium: ion-selective electrode, Roche. Osmolality: method of freezing point depression) will be measured in plasma and in 24-hour urine. These measurements may be taken together utilizing the following formula to calculate the transtubular potassium gradient: $\mathrm{TTPG} = \frac{[K^+]urine}{[K^+]blood} x \frac{Osmblood}{Osmurine}$ [24].

Immune system

To investigate the effect of prednisolone and dexamethasone on the immune system, multiple entities will be investigated. First, absolute leukocyte, granulocyte, and monocyte counts will routinely be performed using flow cytometry. Second, during each study visit peripheral blood mononuclear cells will be isolated utilizing Leucosep tubes (227288, Greiner Bio-one, Kremsmünster, Austria). After isolation peripheral blood mononuclear cells will be aliquoted and placed into isopropanol containers and put into liquid nitrogen for long-term storage.

Lastly, to assess the influence of corticosteroids on a gene expression level, 10ml PAXgene tubes will be collected each visit. PAXgene tubes allow for immediate stabilization of intracellular RNA, thereby facilitating reproducible and accurate gene expression data.

Pharmacokinetic measurements

Population specific pharmacokinetic models and limited sampling strategy were developed to assess the pharmacokinetic parameters of both prednisolone and dexamethasone (MwPharm version 3.81 (Mediware, Zuidhorn, The Netherlands)). MWPharm parameterized a population pharmacokinetic model, originating from literature values [25]. Population pharmacokinetic models of prednisolone and dexamethasone were described with the following parameters (±SD): bioavailability of 82±13% and 86±5%, absorption constant of 1.6±0.1 h-1 and 0.6±0.0 h-1, volume of distribution of 1.5±0.2L/kg and 2.0±0.5 L/kg, and elimination constant of 0.169±0.033 h-1 and 0.154±0.026 h-1, respectively. Furthermore, Monte Carlo analyses were used to develop the limited sample strategy. In these analyses, 1000 patients were simulated for both dosages of prednisolone and dexamethasone. The area under the curve (AUC) was estimated based on 4 points sampling protocol. Performance criteria were set at a R value of >0.95 and a relative root mean squared error of <15%, table 3 [26].

Table 3. Results of the Monte Carlo analyses for the proposed scheme of four sampling points

	% RMSE
Prednisolone - 7.5 mg	3.34
Prednisolone - 30 mg	2.60
Dexamethasone – 1.125 mg	14.1
Dexamethasone – 4.5 mg	4.66

% RMSE, relative root mean squared error

As a result of these calculations, blood samples will be drawn at three time points, namely before, 3 hours after, and 4 hours after ingestion of the study medication on the 7th day. Furthermore, participants are asked to collect saliva at four time points, with the first three time points corresponding to the blood samples and the fourth 7 to 11 hours after ingestion of the last study medication. Plasma cortisol measurements will be performed using validated LC-MS/MS method [27]. Prednisolone and dexamethasone levels in both plasma and saliva will be measured by isotope dilution LC–MS/MS. Cortisol binding globulin (CBG) will be determined by a radioimmuno-assay, and albumin will be measured using the brome cresol green method on a Roche Modular ISE/P. Individual pharmacokinetic parameters will be calculated by maximum a posteriori Bayesian estimation, essentially performed as described by Werumeus Buning [28]. Total body clearance, volume of distribution, $t_{1/2}$, maximum concentration, and AUC will be calculated for all interventions in each individual. Lastly, CYP3A4 and CYP3A5 polymorphisms will be taken into account, as these genetic variations have an important contribution to inter-individual pharmacokinetic variability.

Anthropometrical and metabolic parameters

Anthropometry measurements will include body length, body weight, waist circumference, and hip circumference. Body weight (kg) will be measured without shoes and outer clothing utilizing a calibrated digital measuring scale (seca 877, seca, Hamburg, Germany). Height (cm) will be measured using a wall-secured stadiometer. Waist and hip circumference (cm) will be calculated using a measuring tape roll with standardized retraction mechanism. Waist circumference will be measured mid-way between the lowest rib and the iliac crest with the participant in standing position. Hip circumference will be determined at the maximum circumference over the trochanter major. All anthropometry measurements will be assessed twice after which the average will be utilized in further analyses.

To assess metabolic function and potential changes during corticosteroid use, we will perform an in-depth analysis of the glucose metabolism and lipid profiles. First, fasting glucose levels will be measured using the Roche P Analyzer and fasting insulin levels and c-peptide levels will be measured utilizing a luminescence-immunoassay (Alinity, Abbot, Abbott Park, Illinois, USA). For glucagon-like peptide-1 (GLP-1) special blood collection tubes will be utilized containing K_2EDTA and a proprietary cocktail which includes esterase inhibitors, dipeptidyl peptidase-4 and other protease-inhibitors (P800 Blood Collection Tube, BD Vacutainer®, Franklin Lakes, NJ, USA). To measure active GLP-1 concentrations, commercially available enzyme-linked immunosorbent assay kit (IBL International (Hamburg, Germany) JP27784) will be utilized.

To further investigate the glucose metabolism, a 75-g oral glucose tolerance test will be performed during all study visits. Venous blood samples will be collected before ingestion and at 30, 60, 90, and 120 minutes after ingestion for measurements of glucose, insulin, C-peptide and GLP-1. All glucose samples will be transported to the clinical laboratory immediately after collection to prevent a decay in the glucose levels due to a delay in preanalytical handling (see table 2) [29].

Furthermore, all samples used to determine lipid levels will be collected after an 8-hour overnight fast. The measurement of total cholesterol, low-density lipoprotein, high density lipoprotein, and triglyceride levels will be performed by our in-hospital routine laboratory. Similarly, for measurement of non-esterified fatty acids, fasting blood samples will be collected and will be analyzed utilizing an enzymatic endpoint method (Diasys kit, Roche, Rotkreuz, Switzerland).

Neurocognitive function

A battery of six standardized cognitive tests, as provided by CanTab Cognitive and Psychological test (CANTAB® (Cognitive assessment software) Cambridge Cognition 2019), covering attention, memory and executive functions will be used. We will use the One Touch Stockings of Cambridge for planning, Paired Associates Learning for visual episodic memory, Rapid Visual Information Processing to test sustained attention, Reaction Time to assess processing and psychomotor speed, and the Motor Screening Task to measure sensorimotor function and comprehension. Practice effects are minimized because this test battery provides parallel modes and stimuli randomization.

Questionnaires

At each study visit participants are asked to complete following questionnaires. The 36-Item Short Form Health Survey (SF-36) is a generic and reliable instrument reflecting 8 domains of health, namely physical functioning, physical role, pain, general health, vitality, social function, emotional role, and mental health [30,31]. The Patient Health Questionnaire-15 (PHQ-15) will be utilized to assess the presences and frequency of adverse events as it is a valuable tool for the detection of somatoform disorders [32]. The Medication Adherence Report Scale (MARS-5) is a short questionnaire measuring participants adherence to the study medication and demonstrates acceptable reliability and validity [33]. The Short Questionnaire to Assess Health-enhancing physical activity (SQUASH) is a valid and reliable questionnaire to assess physical activity levels and contains questions about habitual activities with respect to occupation, leisure time, household, transportation means, and other daily activities [34]. Lastly, as food intake, specifically salt intake, can have an influence on blood pressure and other secondary outcome measures, participants will be asked to complete a 3-day food diary [35].

Biomarkers and other endpoints

Due to the difference in mineralocorticoid effects of prednisolone and dexamethasone, it can be hypothesized that this difference may translate into a difference in blood pressure between prednisolone or dexamethasone treatment. Therefore, blood pressure (mmHg) will be measured according to a standardized clinical protocol using an automated device (Omron M2 Basic, Hoofddorp, The Netherlands). Participants will be seated for at least 15 minutes before blood pressure is measured. Then blood pressure and heart rate are measured three times with a 30 seconds interval.

Hand grip strength will be measured using a Jamar Hydraulic Hand Dynamometer (Patterson Medical JAMAR 5030J1, Warrenville, Canada) as describe previously [36,37]. To measure total body muscle mass, 24h urinary creatinine excretion rate will be utilized as it is an excellent and inexpensive measure of muscle mass [38]. Lastly, osteocalcin will be assessed using electrochemiluminescence immunoassay (Cobas E, Roche, Rotkreuz, Switzerland) as it has been linked to physiological processes such as the glucose metabolism [39].

Assignment of interventions

After enrollment by the study physician (SS or AV), the participant is randomized to start with either prednisolone or dexamethasone in a 1:1 ratio. Randomization will be done by the trial pharmacist of the UMCG in accordance with a pre-specified allocation sequence. Randomization is done using a four-block randomization without stratification. The allocation sequence is stored on a secure network station of the pharmacy of the UMCG.

As the CORE study is designed as a double-blind trial, study participants, study physicians, and principle investigators will be blinded. The blinding is guaranteed by the use of identical study medication capsules and medication labels (Apotheek A15, Gorinchem, The Netherlands). The trial pharmacist who will perform the randomization, will be aware of the

intervention assignment. Unblinding will only be done when a serious adverse event (SAE) occurs, which requires the specific knowledge of the used study medication or when the entire trial is completed. Outcomes will be assessed in a unblinded manor.

Data collection, management, and analysis

Once a participant has given written informed consent, the study will consist of a screening visit and 5 study visits. The latter are a baseline visit, after the low dose of the first intervention, after the high dose of the first intervention, and after the low dose and after the high dose of the second intervention. In principle, all study visits are identical with the exception of the baseline visit where no pharmacological endpoints will be assessed. All data will be collected by two trained study physicians (SPS and AV).

All data, including the questionnaires, will be stored using REDCap (REDCap, Vanderbilt University Medical Center, Nashville, TN, USA). All entered data are double checked by both study physicians. Due to the low risk associated with the study interventions no data monitoring safety board was required. The study will however be intensively monitored, according to the guideline "Quality Assurance of research involving human subjects 2.0" of "The Netherlands Federation of University Medical Centers" [40]. The safety will be assessed in two ways. First, as it is undesirable to use exogenous corticosteroids whilst having an active infection, all participants will be checked for any symptoms (including vital signs, physical examination, and laboratory infection parameters) of an active infection during all study visits. Second, all adverse events, including potential SAE, will be documented and the frequency of all adverse events will therefore be deemed a safety measure.

To ensure confidentiality, all participants will receive a unique identification code, which can only be decoded with a separately stored identification file. As in accordance with

the trial information and consent form, participant information is only accessible to the study physician and study monitor, and in case of a SAE may be provided to the trial pharmacist.

Sample size and statistical analyses

To date, no modern day randomized cross-over trials investigating the effects prednisolone and dexamethasone on the HPA-axis (or other endpoints) in healthy individuals are available. Hence, the number of participants which will be included in the CORE study, is based on the scientific guideline of the European Medicines Agency regarding bio-equivalency studies which states that bio-equivalence studies should not include less than 12 subjects [41]. Because males and females clinically differ in terms of circulating levels of oestrogens and corresponding CBG levels, we deemed it necessary to included 12 male and 12 female participants. If drop-out cannot be prevented, new volunteers will be included to ensure adherence to the scientific guideline of the European Medicines Agency.

As the anticipated duration of the trial is expected to be limited no interim analyses will be performed. The newest versions of IBM Statistics SPSS (IBM Inc. Chicago, IL, USA), GraphPad Prism (La Jolla, CA, USA), STATA (STATA Corp., TX, USA), and/or R (Vienna, Austria) will be used for statistical analyses. Demographic data will be presented as median [interquartile range]. To compare paired outcomes, Wilcoxon Signed Ranks Test will be utilized. To compare unpaired data, a Mann-Whitney U-test will be performed. The two-tailed alpha level of <0.05 will be considered statistical significant.

Trial status

The CORE study has started on the 4th of March 2021. On 1st of January 2022 fifteen participants have concluded the study. A total inclusion time of one year and three months was anticipated, however due to the COVID-19 pandemic the complementing vaccination

campaign, the study inclusion is delayed. The extent of the delay is at this moment still unclear.

Ethics and dissemination

The CORE study is conducted according to the principles of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO, The Netherlands). The current study has been approved by the Medical Ethical Committee of the UMCG, The Netherlands (METC 2020.398) on the 18th of January 2021, has been registered on ClinicalTrials.gov (Identifier: NCT04733144), and in the Dutch trial registry (NL9138). Potential protocol amendments will be submitted to the Medical Ethical Committee for review and subsequently distributed to volunteers. Potential participants need to actively seek contact with the investigators and when interested will receive written information. Prior to obtaining informed consent, research staff will explain the aim of the study and all study procedures to the volunteers. Additionally, the research staff will explain that participation is voluntary and that participants are able to withdraw their consent at any given point in time. If the potential participant has no further questions, written informed consent will be obtained from all volunteers by a study physician (SS or AV). Simultaneously, participants are asked if collected data may be used for ancillary studies and if in agreement provide written informed consent. Participants will receive a financial compensation of € 500,-. A full SPIRIT statement checklist can be found in the supplemental material.

This study will be submitted for publication in peer reviewed journals and oral presentations at (inter)national conferences. Authorships will be determined based on the International Committee of Medical Journal Editors guidelines. Raw data will be available upon reasonable request in de-identified form.

Discussion

This article describes the rationale and design of the CORE study which is a randomized, double-blind, cross-over trial investigating the clinical bio-equivalence and dose response of prednisolone and dexamethasone with regard to various physiological systems of the human body. Within this design, the CORE study will include 12 healthy men and 12 healthy women, to receive 7.5 mg prednisolone/1.125 mg dexamethasone and 30 mg prednisolone/4.5 mg dexamethasone all for one week in random order. Data will be collected to evaluate hormonal axes, immunological status, metabolic pathways, pharmacokinetic parameters, and other organ systems with state-of-the-art laboratory techniques.

Although prednisolone and dexamethasone are already widely used in clinical practice, well-validated clinical bio-equivalence data are lacking. The CORE study will help to gain new insight into the comparability between the two medications and improve the existing pharmacodynamic data. By investigating outcome measurements in a cross-over and double-blind fashion, in-depth information regarding the system specific effects of prednisolone and dexamethasone will be gained whilst taking inter-individual differences into account. Another strength of the CORE study the selected dosage and treatment duration reflect clinical practice. This will aid translating the outcomes of the CORE study to routine clinical practice. A limitation of the current study is the absence of a placebo arm. Inclusion of a placebo intervention, however, may result in a substantial increase of the study duration, and may subsequently result in negative effect on the inclusion rate. As a result, a baseline study visit was implemented to serve as reference point. Another limitation could be the relative low number of participants. Nevertheless, the number of included participants is in concordance with current guidelines of bio-equivalence study of the European Medicines Agency and is even double the number of minimal requirement of subjects, to allow for subgroup analyses based on age and sex.

Lastly, this study investigates the effects of prednisolone and dexamethasone in healthy volunteers. However, in various disease states some aspects of glucocorticoid action could change in a disease specific manner. In order to draw conclusions on glucocorticoid action in specific disease states, further research is needed.

In conclusion, the CORE study has the potential to improve the current understanding of the most widely used corticosteroids and may therefore aid various clinicians in clinical decision making, including general practitioners, endocrinologists, nephrologists, rheumatologists and many more.

References

1 Benedek TG. History of the development of corticosteroid therapy. *Clin Exp Rheumatol* 2011;**29**.

2 Fardet L, Petersen I, Nazareth I. Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years. *Rheumatology (Oxford)* 2011;**50**:1982–90.

3 Furst DE, Saag KG. Determinants of glucocorticoid dosing. UpToDate, Post TW (Ed), UpToDate, Waltham, MA. https://www.uptodate.com/contents/determinants-of-glucocorticoid-dosing?sectionName=BIOEQUIVALENCE AND BIOAVAILABILITY&topicRef=124&anchor=H2&source=see_link#H2 (accessed 9 Dec 2021).

4 Herzog H, Nobile A, Tolksdorf S, *et al.* New Antiarthritic Steroids. *Science.* 1955;**121**.

5 Boland EW. 16a-Methyl corticosteroids; a new series of anti-inflammatory compounds; clinical appraisal of their antirheumatic potencies. *Calif Med* 1958;**88**:417–22.

6 Krasowski MD, Drees D, Morris CS, *et al.* Cross-reactivity of steroid hormone immunoassays: Clinical significance and two-dimensional molecular similarity prediction. *BMC Clin Pathol* 2014;**14**:1–13.

7 Peters WP, Holland JF, Senn H, *et al.* Corticosteroid administration and localized leukocyte mobilization in man. *N Engl J Med* 1972;**286**:342–5.

8 Meikle AW, Tyler FH. Potency and duration of action of glucocorticoids. Effects of hydrocortisone, prednisone and dexamethasone on human pituitary-adrenal function. *Am J Med* 1977;**63**:200–7.

9 Melby JC. Systemic corticosteroid therapy: pharmacology and endocrinologic considerations. *Ann Intern Med* 1974;**81**:505–12.

10 Mager DE, Lin SX, Blum RA, *et al.* Dose Equivalency Evaluation of Major Corticosteroids: Pharmacokinetics and Cell Trafficking and Cortisol Dynamics. *J Clin Pharmacol* 2003;**43**:1216–27.

11 Schimmer BP, Funder JW. ACTH, Adrenal Steroids, and Pharmacology of the Adrenal Cortex. In: Brunton LL, Chabner BA, Knollmann BC, eds. *Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 12e*. New York, NY: : McGraw-Hill Education 2015.

12 Diederich S, Eigendorff E, Burkhardt P, *et al.* 11β-hydroxysteroid dehydrogenase types 1 and 2: An important pharmacokinetic determinant for the activity of synthetic mineralo- and glucocorticoids. *J Clin Endocrinol Metab* 2002;**87**:5695–701.

13 Nicolaides NC, Charmandari E, Chrousos GP, *et al.* Recent advances in the molecular mechanisms determining tissue sensitivity to glucocorticoids: Novel mutations,

circadian rhythm and ligand-induced repression of the human glucocorticoid receptor. *BMC Endocr Disord* 2014;**14**:1–12.

14 Kino T. Tissue Glucocorticoid Sensitivity: Beyond Stochastic Regulation on the Diverse Actions of Glucocorticoids. *Horm Metab Res* 2007;**39**:420–4.

15 Czock D, Keller F, Rasche FM, *et al.* Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clin Pharmacokinet* 2005;**44**:61–98.

16 Werumeus Buning J, Touw DJ, Brummelman P, *et al.* Pharmacokinetics of oral hydrocortisone - Results and implications from a randomized controlled trial. *Metabolism* 2017;**71**:7–16.

17 Vulto A, Minović I, de Vries L V., *et al.* Endogenous urinary glucocorticoid metabolites and mortality in prednisolone-treated renal transplant recipients. *Clin Transplant* 2020;**34**:1–14.

18 Furst DE, Saag KG. Glucocorticoid withdrawal. UpToDate, Post TW (Ed), UpToDate, Waltham, MA. (Accessed 9 Dec 2021)

19 Prete A, Bancos I. Glucocorticoid induced adrenal insufficiency. *Br Med J* 2021;**374**:n.1380.

20 Jong WHAD, Buitenwerf E, Pranger AT, *et al.* Determination of reference intervals for urinary steroid profiling using a newly validated GC-MS/MS method. *Clin Chem Lab Med* 2017;**56**:103–12.

21 de Vries L V., de Jong WHA, Touw DJ, *et al.* Twenty-four hour urinary cortisol excretion and the metabolic syndrome in prednisolone-treated renal transplant recipients. *Steroids* 2017;**127**:31–9.

22 van der Veen A, van Faassen M, de Jong WHA, *et al.* Development and validation of a LC-MS/MS method for the establishment of reference intervals and biological variation for five plasma steroid hormones. *Clin Biochem* 2019;**68**:15–23.

23 Van Der Gugten JG, Crawford M, Grant RP, *et al.* Supported liquid extraction offers improved sample preparation for aldosterone analysis by liquid chromatography tandem mass spectrometry. *J Clin Pathol* 2012;**65**:1045–8.

24 West ML, Bendz O, Chen CB, *et al.* Development of a test to evaluate the transtubular potassium concentration gradient in the cortical collecting duct in vivo. *Miner Electrolyte Metab* 1986;**12**:226–33.

25 Prednisolone and Dexamethasone. In: DrugDex [database on the Internet]. Greenwood Village (CO): IBM Corporation; 2020 [cited 09-11-2020]. Available from: www.micromedexsolutions.com. Subscription required to view.

26 Van den Elsen SHJ, Sturkenboom MGG, Van't Boveneind-Vrubleuskaya N, *et al.* Population pharmacokinetic model and limited sampling strategies for personalized dosing of levofloxacin in tuberculosis patients. *Antimicrob Agents Chemother* 2018;**62**.

27 van Faassen M, Bischoff R, Kema I. Relationship between plasma and salivary melatonin and cortisol investigated by LC-MS/MS. *Clin Chem Lab Med* 2017;**55**:1340–948.

28 Werumeus Buning J, Touw DJ, Brummelman P, *et al.* Pharmacokinetics of oral hydrocortisone - Results and implications from a randomized controlled trial. *Metabolism* 2017;**71**:7–16.

29 Potter JM, Hickman PE, Oakman C, *et al.* Strict preanalytical oral glucose tolerance test blood sample handling is essential for diagnosing gestational diabetes mellitus. *Diabetes Care* 2020;**43**:1438–41.

30 Brazier JE, Harper R, Jones NMB, *et al.* Validating the SF-36 health survey questionnaire: New outcome measure for primary care. *Br Med J* 1992;**305**:160–4.

31 Cech DJ, Martin S "Tink". Chapter 5 - Evaluation of Function, Activity, and Participation. In: Cech DJ, Martin S "Tink", eds. *Functional Movement Development Across the Life Span (Third Edition)*. Saint Louis: : W.B. Saunders 2012. 88–104.

32 Kroenke K, Spitzer RL, Williams JBW. The PHQ-15: Validity of a new measure for evaluating the severity of somatic symptoms. *Psychosom Med* 2002;**64**:258–66.

33 Chan AHY, Horne R, Hankins M, *et al.* The Medication Adherence Report Scale: A measurement tool for eliciting patients' reports of nonadherence. *Br J Clin Pharmacol* 2020;**86**:1281–8.

34 Wendel-Vos GCW, Schuit AJ, Saris WHM, *et al.* Reproducibility and relative validity of the short questionnaire to assess health-enhancing physical activity. *J Clin Epidemiol* 2003;**56**:1163–9.

35 Yang YJ, Kim MK, Hwang SH, *et al.* Relative validities of 3-day food records and the food frequency questionnaire. *Nutr Res Pract* 2010;**4**:142.

36 Angst F, Drerup S, Werle S, *et al.* Prediction of grip and key pinch strength in 978 healthy subjects. *BMC Musculoskelet Disord* 2010;**11**:2–7.

37 Eisenga MF, Gomes-Neto AW, Van Londen M, *et al.* Rationale and design of TransplantLines: A prospective cohort study and biobank of solid organ transplant recipients. *BMJ Open* 2018;**8**:1–13.

38 Heymsfield SB, Arteaga C, McManus C, *et al.* Measurement of muscle mass in humans: validity of the 24-hour urinary creatinine method. *Am J Clin Nutr* 1983;**37**:478–94.

39 Moser SC, van der Eerden BCJ. Osteocalcin — A versatile bone-derived hormone. *Front Endocrinol (Lausanne)* 2019;**10**:4–9.

40 Guideline Quality assurance of research involving human subjects. https://www.nfu.nl/sites/default/files/2021-

01/21.00024_Guideline_Quality_assurance_of_research_involving_human_subjects_dec20_0.pdf. (accessed 30 Mar 2021).

41 European Medicines Agency. GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE. 2010. Doc. Ref.: CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **

Declarations

Acknowledgements

We would like to acknowledge the help of Tanja Zijp, PharmD with all pharmacokinetics timepoint calculations. In addition, we would like to extent our gratitude to Elisabeth Raveling-Eelsing for performing all peripheral blood mononuclear cell isolations.

Author contributions

SJB and APvB are the principal investigators and devised the original draft of the study design. MJV and IPK have provided valuable input on the utilized laboratory measurement and methods. MNK has aided in critical review of the current manuscript. AR has facilitated the collection of the immunological study endpoints and provided vital knowledge to the design of the study. DJT has provided crucial input with regard to the pharmacological aspects of the interventions and the study design. SPS and AV are collecting the data and wrote the first draft of the design paper. All authors have read and approved the manuscript.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Institutional Review Board Statement

The CORE study has been approved by the Medical Ethics Committee of the UMCG (2020.398) on 18th of January 2021. The trial was designed and implemented in accordance with the Good Clinical Practice guidelines and follows the International Council on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human

Use (including European Directive 2001/20/EC) and adherence to the ethical principles as laid down in the Declaration of Helsinki, Brazil, October 2013.

Informed Consent Statement

All participants will have to provide written informed consent prior to the enrollment in the trial. The informed consent contains a subsection on the storage and future use of any bodily materials collected during the trial.

Competing interest

The authors declare no conflict of interest. Furthermore, the sponsors had no role in the design, execution, interpretation, or writing of the study.

Figure legend

Figure 1. IC, Informed consent; B, baseline; RND, randomization; WO, wash-out; w, weeks

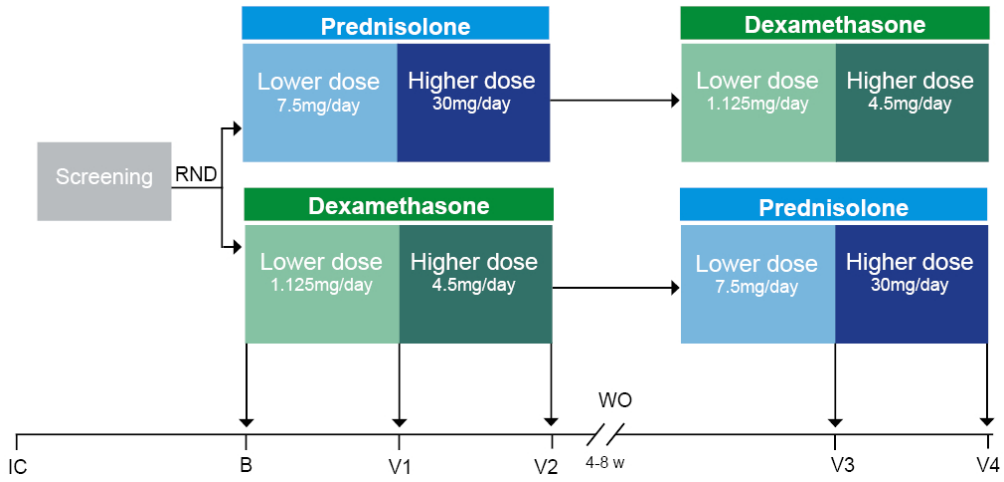

Figure 1. IC, Informed consent; B, baseline; RND, randomization; WO, wash-out; w, weeks

672x320mm (38 x 38 DPI)

Supplemental Material

Rationale and Design of the CORE (COrticosteroids REvised) study: A Randomized Cross-over Clinical Trial of Prednisolone versus Dexamethasone

Suzanne P. Stam, MD[1]*, Annet Vulto, MD[2]*, Michel J. Vos, PhD[3], Michiel N. Kerstens, MD,PhD[2], Abraham Rutgers, MD, PhD[4], Ido P. Kema, PhD[3], Daan J. Touw, PharmD, PhD[5], Stephan J.L. Bakker MD,PhD[1], André P. van Beek, MD,PhD[2]

* contributed equally

[1] Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[2] Department of Internal Medicine, Division of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[3] Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands

[4] Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands.

[5] Department of Clinical Pharmacy and Pharmacology, University of Groningen, Groningen, University Medical Center Groningen, Groningen, 9713 GZ Groningen, The Netherlands

Corresponding author:

A.P. van Beek, MD PhD

Department of Internal Medicine, Division of Endocrinology

University Medical Center Groningen

P.O. Box 30.001, 9700 RB Groningen, the Netherlands

Phone: 0031 050 361 0388, Email: a.p.van.beek@umcg.nl

Appendix A

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	**1**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	**2, 5, 11**
	2b	All items from the World Health Organization Trial Registration Data Set	**N.A.**
Protocol version	3	Date and version identifier	**2**
Funding	4	Sources and types of financial, material, and other support	**2, 26**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	**1-3, 26**
	5b	Name and contact information for the trial sponsor	**3**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	**N.A.**
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	**N.A.**
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	**7-8**
	6b	Explanation for choice of comparators	**7**
Objectives	7	Specific objectives or hypotheses	**8**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	**9**

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	**9**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	**9,10,21**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	**12**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	**21**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	**12,19**
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	**10**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	**12-20**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	**9, 21, See fig 1**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	**22**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	**9,11**
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	**20**

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	**20**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	**20**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	**20,21**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	**21**
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	**12-20**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	**22**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	**21,22**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	**22**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	**N.A.**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	**22**
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	**21**

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	**N.A.**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	**19,22**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	**21**
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	**11**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	**11**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	**11**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	**11**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	**21,22**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	**27**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	**N.A.**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	**11**
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	**22**
	31b	Authorship eligibility guidelines and any intended use of professional writers	**22**
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	**22**

Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	**App B**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	**14**

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Appendix B

Toestemmingsformulier proefpersoon

Een nieuwe blik op corticosteroïden

- Ik heb de informatiebrief gelezen. Ook kon ik vragen stellen. Mijn vragen zijn voldoende beantwoord. Ik had genoeg tijd om te beslissen of ik meedoe.
- Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen of te stoppen met het onderzoek. Daarvoor hoef ik geen reden te geven.
- Ik geef toestemming voor het informeren van mijn huisarts dat ik meedoe aan dit onderzoek
- Ik geef toestemming voor het verzamelen en gebruiken van mijn gegevens, bloedmonsters en lichaamsmateriaal voor de beantwoording van de onderzoeksvraag in dit onderzoek
- Ik weet dat voor de controle van het onderzoek sommige mensen toegang tot al mijn gegevens kunnen krijgen. Die mensen staan vermeld in deze informatiebrief. Ik geef toestemming voor die inzage door deze personen.
- Ik geef toestemming voor het informeren van mijn huisarts en/of behandelend specialist van onverwachte bevindingen die van belang (kunnen) zijn voor mijn gezondheid.
- Ik weet dat ik niet zwanger mag worden tijdens het onderzoek.
- Vrouwelijke deelneemsters: de onderzoeker heeft het gebruik van anticonceptie met mij besproken.
- Ik geef □ **wel**

  □ **geen**

  toestemming om mijn persoonsgegevens langer te bewaren en te gebruiken voor toekomstig onderzoek op het gebied van de onderzoeksmedicatie.
- Ik geef □ **wel**

  □ **geen**

  toestemming om mijn lichaamsmateriaal na dit onderzoek te bewaren en om dit later nog voor meer onderzoek te gebruiken, zoals in de informatiebrief staat.
- Ik geef □ **wel**

  □ **geen**

  toestemming om mij na dit onderzoek opnieuw te benaderen voor een vervolgonderzoek.
- Ik wil □ **wel**

  □ **niet**

  geïnformeerd worden over welke behandeling ik heb gehad/in welke groep ik zat.

- **Ik wil meedoen aan dit onderzoek.**

Naam proefpersoon:

Handtekening: Datum : __ / __ / __

Ik verklaar dat ik deze proefpersoon volledig heb geïnformeerd over het genoemde onderzoek.

Als er tijdens het onderzoek informatie bekend wordt die de toestemming van de proefpersoon zou kunnen beïnvloeden, dan breng ik hem/haar daarvan tijdig op de hoogte.

Naam onderzoeker (of diens vertegenwoordiger):

Handtekening: Datum: __ / __ / __

---

* Doorhalen wat niet van toepassing is.

De proefpersoon krijgt een volledige informatiebrief mee, samen met een getekende versie van het toestemmingsformulier.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	**1**
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	**2, 5, 11**
	2b	All items from the World Health Organization Trial Registration Data Set	**N.A.**
Protocol version	3	Date and version identifier	**2**
Funding	4	Sources and types of financial, material, and other support	**2, 26**
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	**1-3, 26**
	5b	Name and contact information for the trial sponsor	**3**
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	**N.A.**
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	**N.A.**
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	**7-8**
	6b	Explanation for choice of comparators	**7**
Objectives	7	Specific objectives or hypotheses	**8**
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	**9**

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	**9**
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	**9,10,21**
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	**12**
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	**21**
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	**12,19**
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	**10**
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	**12-20**
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	**9, 21, See fig 1**
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	**22**
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	**9,11**
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	**20**

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	**20**
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	**20**
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	**20,21**
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	**21**
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	**12-20**
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	**22**
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	**21,22**
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	**22**
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	**N.A.**
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	**22**
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	**21**

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	**N.A.**
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	**19,22**
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	**21**
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	**11**
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	**11**
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	**11**
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	**11**
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	**21,22**
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	**27**
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	**N.A.**
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	**11**
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	**22**
	31b	Authorship eligibility guidelines and any intended use of professional writers	**22**
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	**22**

Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	**App B**
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	**14**

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.