Protovol (Version 3.1 – updated 23MAY2020)
Spermidine Anti-Hypertension Study (SMARTEST)

Effect of spermidine on arterial blood pressure in hypertensive patients

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Contents

1 Background

1.1 Rationale of the study

Hypertension is the leading risk factor for cardiovascular disease and mortality[1]. Standard pharmacotherapy, albeit fairly effective, is far from being optimized and adequate blood pressure control can be maintained only in a limited number of hypertensive patients. In line with this clear medical need, we recently discovered that supplementation of the natural polyamine spermidine effectively delays the development of experimental hypertension and the associated heart failure[2]. Importantly, higher dietary intake of spermidine correlates with reduced blood pressure[2], as well as decreased overall mortality[3] in human cohort studies. That said, randomized clinical trials examining the anti-hypertensive effect of spermidine in humans are still lacking.

Relevance to the international status of research in the field

Despite the widely recognized benefits of reducing elevated blood pressure, hypertension is still the leading cause of many life-threatening cardiovascular diseases, such as stroke, coronary artery disease as well as heart and renal failure[4, 5]. The current prevalence of hypertension is devastating as the number of hypertensive individuals, *i.e.*, systolic blood pressure (SBP) of 140 mmHg or higher, has almost doubled between 1990 and 2015, and now impacts 874 million patients[5]. In fact, non-optimal systolic blood pressure is the major contributing risk factor to morbidity and all-cause mortality worldwide, causing a loss of 212 million healthy life years and 9.4 million deaths annually[6]. Thus, hypertension poses a massive burden on both the public health and the economy, as it is the costliest cardiovascular disease[7].

This unprecedented prevalence of hypertension is influenced by the growing elderly population due to increased life expectancy. In addition, habitual risk factors, such as increased caloric and salt intake combined with a sedentary life-style, have facilitated=fostered the age-related surge in blood pressures. That being said, very few interventions have been successful in achieving extended and long-lasting control with any of these risk factors. For decades, studies have extensively searched for more effective interventions, but anti-hypertensive pharmacotherapy remains the most important means (I would rather say tool) for reducing high blood pressures. However, anti-hypertensive pharmaceuticals, even though being fairly effective and accessible at an affordable price, are far from ideal. Indeed, although a great majority (87.5%) of those diagnosed with hypertension receive regular pharmacological therapies, only a minority (32.5%) show controlled blood pressures (SBP < 140 mmHg), despite the fact that nearly one third of them already receive 2 or more drugs[8]. Using Austria as an example, controlled blood pressure is achieved in only 17% of hypertensive patients[9] and data from other European countries reveals

comparable results. **Therefore, further investigation is necessary to develop better therapies for managing hypertension and avoiding hypertension-related complications.**

In this regard, spermidine ─ a natural caloric restriction mimetic ─ delays the onset of hypertension in a strain of rats sensitive to salt intake[2]. Our initial goal of preventing these animals from developing fatal heart failure induced by severe hypertension led us to discover a direct effect of spermidine on hypertension[2]. This finding was further corroborated by an epidemiological study in humans (BRUNECK study), where we observed that higher dietary intake of spermidine correlated with reduced blood pressure and decreased cardiovascular events[2]. Interestingly, spermidine supplementation in elderly subjects coincided with a trend towards reduced blood pressure (a phase I safety trial)[10]. Even in the absence of hypertension, preclinical evidence supports vascular benefits of spermidine treatment, which was shown to reduce age-related vascular stiffness and endothelial dysfunction[11].

In general, the beneficial actions of spermidine are strongly linked to its ability to induce autophagy[2, 11, 12], a key process that is indispensable for tissue homeostasis in both health and disease[13]. Based on the existing evidence, it is reasonable to hypothesize that inducing endothelial autophagy enhances vascular function and, thus, contributes to the reduction in blood pressure. Indeed, a causal implication of autophagy in the blood pressure-reducing effect of spermidine is currently being tested in our ongoing experimental studies.

Besides boosting autophagy, spermidine has been shown to stimulate and improve immune cell functions in several pathophysiological settings[12, 14-16]. Given the accumulating evidence supporting a critical role of immune cells in the development of hypertension[17] and cardiovascular disease (CVD)[18], it is possible that spermidine also modulates elevated blood pressures via anti-inflammatory and immune cell-specific functions.

Another plausible and autophagy-independent mechanism through which exogenous spermidine may reduce high blood pressures is through increasing the bioavailability of L-arginine[2, 19], a nitric oxide (NO) precursor. An increase in spermidine intake subsequently results in an increase in circulating and tissue levels of polyamines, which in turn decreases the *de novo* polyamine synthesis from their precursor L-arginine. Therefore, L-arginine can be primarily directed for increased production of the vasodilator NO.

Inspite of the initial epidemiological indication that spermidine effect may differ between men and women[2], spermidine – like caloric restriction and other caloric restriction mimetics (CRMs) – extends life span of several animal species in a sex-independent manner (for details – see Table S1 in this review[12]). More importantly, the first prospective trial administering a spermidine-rich

wheat-germ extract to elderly people with subjective cognitive decline revealed no sex-based differences in any of the tested parameters[12].

In summary, growing experimental and epidemiological evidence suggests that spermidine preserves vascular health and delays the onset of hypertension as a result of common risk factors, including aging and salt sensitivity. However, whether spermidine can be used for managing hypertension as an adjuvant to the existing pharmacotherapy in humans is still unknown. In fact, human data obtained from interventional spermidine trials are sparse. Similarly, the mechanisms ─ whether they are autophagy-dependent or independent ─ that underlie cardiovascular benefit of spermidine still require further investigation in humans. **In the proposed project, we are aiming to define an optimal blood pressure management strategy by using a spermidine-rich plant extract in patients who are not responsive to first-line anti-hypertensive drugs.** This extract has an established safety profile as observed in a randomized, placebo-controlled phase I trial in elderly subjects[10]. **In addition, we will explore potential mechanisms of action for the blood pressure-lowering effect of spermidine.**

Work leading up to this project (preliminary results)

In an experimental pilot study (not published), we examined the therapeutic effect of spermidine supplementation on hypertension using male *Dahl* salt-sensitive rats. After four weeks of a high-salt diet (8% NaCl), 12-week-old *Dahl* rats developed severe hypertension (190-200 mmHg, Fig. 1A) associated with hypertrophy (not shown). All rats then received 'standard' treatment in the form of a diuretic (furosemide, 10 mg/kg body weight, single intraperitoneal injection), as well as a low-salt diet (0.3% NaCl) combined with or without spermidine supplementation (3 mM) in drinking water for the following six weeks. Induced diuresis combined with a change from a high- to low-salt diet resulted in a transient and relatively small reduction in blood pressure, which ultimately returned to the same level it had been before the intraperitoneal administration of the diuretic (Fig. 1A). However, spermidine supplementation resulted in a long-lasting reduction in blood pressure (Fig. 1A) and restored vascular elasticity (Fig. 1B) to levels comparable with those from healthy controls (rats fed only a low-salt diet). In addition, spermidine reduced cardiac hypertrophy and end-diastolic pressure, while enhancing exercise tolerance as indicated by improved running distance and maximum oxygen consumption (Fig. 1C-F). Taken together, this indicates – for the first time – a therapeutic, rather than preventive, effect of spermidine supplementation on salt-induced hypertension.

More importantly, initial data obtained from humans also indicate a potential blood pressure-lowering effect of spermidine, as shown in the SMARTAGE trial in elderly patients with subjective decline in cognitive function[10]. In this trial, spermidine supplementation resulted in a prominent

trend towards decreased systolic blood pressure, despite the moderate dose and low number of subjects involved.

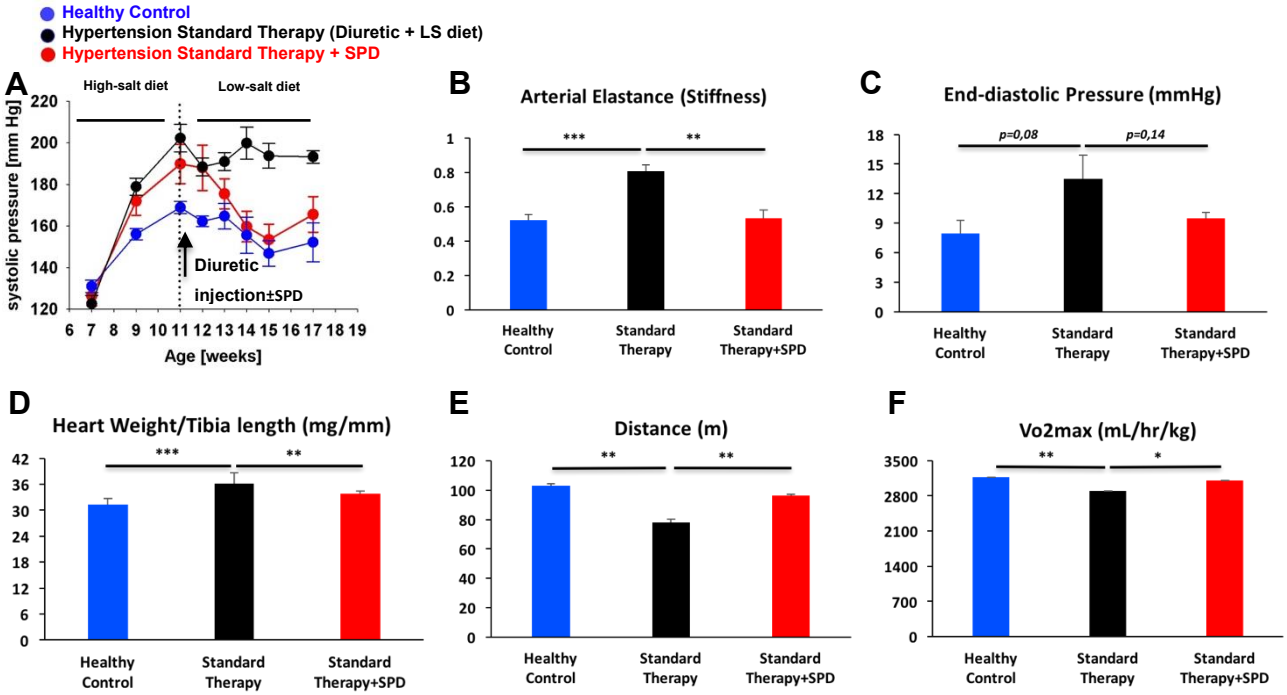

Figure 1. Spermidine potentiates the effects of standard hypertension therapy. Excluding the healthy control group (blue), all animals received a high-salt diet and developed hypertension and cardiac hypertrophy before the onset of therapy, which consisted of the diuretic furosemide and reduced salt intake with or without 3 mM spermidine supplementation in drinking water. Depicted are the mean±S.E.M of (A) non-invasive (tail-cuff-based) systolic blood pressure measurements, invasively (hemodynamically) measured (B) arterial elastance (*i.e.* stiffness) and (C) end-diastolic pressure, (D) heart weight-to-left tibia length ratio as a measure for cardiac hypertrophy, as well as fatigue and exercise tolerance as determined by (E) treadmill run distance and (F) maximum oxygen consumption (VO_2max), respectively. n=5-6 rats/group. *p<0.05, **p<0.01, ***p<0.001 (Bonferroni-corrected ANOVA). Abbreviations: SPD, spermidine; LS, low-salt.

1.2 Objectives

The primary objective of this single-centre, placebo-controlled, double-blind and randomized trial with a crossover design and washout period between treatment arms is to assess the effect of oral spermidine supplementation (4 mg/day) for 8 weeks on systolic blood pressure, as measured by 24h-blood pressure measurement (BPM).

The secondary objective of the study is to examine the effect of spermidine on pulse-wave velocity, ambulatory blood pressure and 6MWT.

As exploratory objectives of the trial, we aim to investigate if certain amino acids and metabolites, as well as markers of autophagy and immune cell profiles, correlate with blood pressure-lowering effects.

1.3 Outcomes

1.3.1 24h-BPM

- Systolic, diastolic, mean, and central blood pressure
- Pulse wave velocity (PWV)
- Augmentation @75 Index (AIx@75)
- Stroke volume
- Peripheral resistance

1.3.2 Laboratory parameters

Spermidine and its metabolites (polyamine profiles will be determined using HPLC-MS/MS from plasma, whole blood, peripheral blood mononuclear cells (PBMCs) and saliva samples), NT-pro-BNP, liver and kidney function, tryptophan, kynurenine, kynurenine acid, 3-hydroxykynurenine, quinolic acid, global arginine bioavailability ratio (GABR: L-Arginin/[Citrulline+Ornithin]), arginase activity (L-Arginine/Ornithine ratio), asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), L-Arginine/ADMA ratio, C-reactive protein (hs-CRP), homoarginine, amino acids and their metabolites, ACTH, (nor-)epinephrine, electrolytes (K^+, Na^+), aldosterone, plasma renin activity, immuno cell profile (WBC, specific leukocyte populations), circulatory cytokines (e.g., IL-6, TNF-alpha, IL-17), markers of autophagy including the lipidation of LC3 (assessed using an *ex vivo* leupeptine-based autophagy flux assay[20]), and p62 protein levels in PBMCs. A total of 30 ml of whole blood will be collected from each patient at every visit.

1.3.3 ECG

Frequency, rhythm, repolarisation times.

1.3.4 Echocardiography

Systolic and diastolic function, left ventricular mass index (LVMI), left atrial volume index (LAVI), flow velocities (E- and A-wave, LVOT); myocardial velocities (e') and strain analysis (longitudinal, circumferential and radial). Also see the protocol for Echo-CRF (Appendix).

1.3.5 Quality of Life (QoL)

Quality of life will be assessed at all visits using the established SF-36 questionnaire.

2 Trial design

2.1 Trial design

The study is designed as a single-centre, placebo-controlled, double-blind and randomized controlled trial with a balanced 2x2 crossover design and a washout period between treatment arms. Specifically, half of the recruited patients will be treated with spermidine for eight weeks, followed by placebo for eight weeks. The other half of patients will first receive placebo and then spermidine. There will be a washout period of four weeks between the first period of eight weeks and second period of eight weeks to separate the two interventions in both arms, which will avert any carryover or residual effects. A balanced 2x2 crossover trial design is ideal for this study for the following two reasons:

(i) Physiological reason: spermidine levels in the body are substantially affected by the diet. Thus, patients with different dietary habits could already have different spermidine levels at baseline (i.e. before starting the trial). Even if patients were randomized into 2 groups correcting for baseline measured circulating spermidine, this wouldn't guarantee that spermidine levels in the body tissues are comparable without confirming via biopsy, not to mention that food intake would need to be strictly controlled and be identical in both arms throughout the trial. For this reason, crossover/repeated measurements are not only more practical, but rather essential for our proposed study. The effect of any other confounding factors (e.g. body mass index, age, smoking, baseline blood pressure, LDL, etc.) will be also reduced to a minimum as every patient will serve as his/her own control.

(ii) Statistical reason: a crossover design is particularly efficient as it provides the highest power-to-sample size ratio. Thus, the study endpoints can be examined with as few patients as possible.

2.2 Schedule of evaluations

The assessments planned for during the study are shown below (Table 1).

Table 1:

Visit number	1	2	3	4	5
Week number	-4 (+/-3 days)	0 (+/-3 days)	8 (+/-3 days)	12 (+/-3 days)	20 (+/-3 days)
Screening	x				
Informed consent	x				
24h-BPM		x	x	x	X
Laboratory analysis		x	x	x	X
ECG		x	x	x	X
Echocardiography		x	x	x	X
SF-36 questionaire		x	x	x	X

2.3 Trial population

The trial population consists of mixed adults from both genders screened during hospitalisation at a medical clinic. Patients selected must present with elevated blood pressure despite being treated with at least two guideline-recommended drugs. This approach secures a trial population with well-established arterial hypertension and ensures that patients will have received baseline treatment based on the guideline recommendations before being enrolled in the proposed study. Therefore,

our study offers an additional novel and innovative strategy for patients following their initial baseline treatment which failed to fully manage their hypertension. Patients who need to be excluded due to systolic blood pressures > 180mmHg will directly be advised to get further treatment at the outpatients clinic and the educational program herz.leben.

Recruitment

Patients with systolic arterial hypertension of more than 150 mmHg during hospitalisation despite being treated with at least two guideline-recommended drugs will be informed of the possibility to participate in the trial. If they consent, patients will be contacted four weeks later by telephone to check for unchanged medication and blood pressure. Patients with persistent arterial hypertension (>150 mmHg), despite being on a consistent medication routine with at least two guideline-recommended anti-hypertensive drugs within the last four weeks, will be invited for a screening (Visit 1) at our hospital. Upon confirmation of sustained high blood pressure (>150 ≤180mmHg) and fulfilment of inclusion and exclusion criteria, patients will again be informed that participation in the trial is voluntary before signing the informed consent form. After signing the ICF, patients will be randomized in a 1:1 ratio and a block size of 46 using the web-based randomizer (https://randomizer.at/) provided by Prof. Andrea Berghold (Institute for Medical Informatics, Statistics and Documentation – IMI, Medical University of Graz). We aim to have both genders equally represented within the trial population. We will perform a gender-specific analysis of clinical and biochemical assessments.

2.4 Inclusion and exclusion criteria

Inclusion criteria

- Able to provide signed and dated informed consent form
- Male or female, 19 to 99 years of age
- Persistant arterial hypertension with systolic blood pressure above 150 mmHg during hospitalisation and the day of randomisation
- Stable anti-hypertensive medication with at least two guideline-recommended anti-hypertensive drugs

Exclusion criteria

- Systolic blood pressure ≥180mmHg on the day of randomisation
- Spermidine intolerance
- Significant renal impairment defined as glomerular filtration rate < 45ml/min
- Insulin-dependent diabetes mellitus (IDDM)
- Wheat allergy or gluten intolerance
- Life expectancy of less than 12 months

- Participation in another clinical trial

2.5 Data management / documentation

Data will be collected on paper-based CRFs. CRFs will remain the property of the study center (Medical University of Graz). Data analysis will be performed using SPSS on the institutional computers of the investigators.

2.6 Sample size considerations

As outlined in 2 and 2.1., the blood pressure-lowering effect of spermidine is well established in animal models. Furthermore, 28 elderly patients with subjective declining cognitive function within the SMARTAGE trial revealed a drop in systolic blood pressure of 10 mmHg (4.5 mmHg more than in the placebo group) upon spermidine (1.2 mg/day) supplementation for three months. In the proposed trial, we will use a three-fold higher dose of spermidine and we will enroll patients with a higher baseline blood pressure (≥150 and ≤180 mmHg systolic BP). Therefore, we expect a reduction of systolic blood pressure of at least 5 mmHg. To this end, we calculated that a total of 38 patients would be sufficient to detect that effect at a power of 80%, assuming a two-tailed α value (type-I error) of 5%, blood pressure variance (SD) of 13 mmHg and within-subject correlation coefficient (r) of 0.65. We expect a drop-out rate of 15% due to the 20-week-long duration of the trial, thus, a total number of 46 patients will be enrolled.

2.7 Follow-up

Study follow-up will be twenty weeks, comprised of two treatment periods of eight weeks and a washout period of four weeks between both treatments.

2.8 Project duration and milestones

We envision that a period of 21 months will be needed to successfully accomplish this study. The recruitment (18 months) is planned as follows:

First patient visit:	February 2020
Last patient enrolled:	June 2021

3 Methods

3.1 24h BPM

During each of the four visits patients will be supplied with an ambulatory blood pressure measurement (ABPM) device (Mobil-O-Graph® PWA, I.E.M. GmbH, Stolberg, Germany). After 24 hours of continuous BPM at their homes, patients will remove the device and return it to the study center via postal service. Mobil-O-Graph® PWA is a well established device to measure central blood pressure and pulse wave velocity used and validated in multiple clinical trials and settings. Despite being a non-invasive technique good accuracy compared to intra-aortic readings has been proven (linear correlation R=0.81, p <0.0001) in 120 patients (see validation data on www.iem.de/en/health-management/studies-validations.html and a bibliography at www.iem.de/files/content/DE/IEMintern/Support/Hintergründe/Bibliography%20Mobil-O-Graph%20Blood%20Pressure%20PWA%202.9.pdf) During the visit on-site blood pressure measurements and a standardized 6MWT will be conducted at every visit.

3.2 Lab analysis

Blood withdrawal will be performed using:

- 4x Lithium-heparinate: 8+7+2+2 ml=19 ml blood
- 1x Serum: 4 ml blood for approx. 2 ml serum
- 1x EDTA: 7 ml blood for approx. 3.5 ml plasma

Blood draws will be performed at each of the four visits (total blood volume taken during the trial will be 120 ml).

In addition to standard clinical assessments (see 2.3.2), the isolation of PBMC will be performed from the whole blood samples and analysed to assess alterations in immune cell populations using state-of-the-art multi-parameter flow cytometry and/or Cy-TOF. In several multi-systemic pathologies, possibly rare, differentiated or non-differentiated hematopoietic cell subpopulations, which can be detected in circulation, have been increasingly recognized as a read-out to determine/distinguish between health and disease status. Advances in single cell-resolved molecular measurement technologies enabled the description of cell population heterogeneity, including rare disease-associated subpopulations. However, the identification of such subpopulations remains a difficult computational task. Therefore, our collaborator Prof. Claassen (ETH Zurich, Switzerland) has developed[21] an artificial intelligence approach that detects disease-associated subpopulations of cells from single-cell proteomic measurements using this CyTOF

techniques[22]. These data will be examined for correlations with systolic and diastolic blood pressures as well as with markers of inflammation employing flow cytometry-based assessment of cytokines. In addition, autophagic flux will be measured using LC3-lipidation quantification in *ex vivo* leupeptin-treated PBMCs[20]) in human leukocytes. Altogether, this will allow us to describe, for the first time, *in vivo* effects of spermidine intervention on human immune cells, inflammatory cytokines, and markers of autophagy.

3.3 Electrocardiography

Conventional 12-lead ECG.

3.4 Echocardiography

Standardized 2D-echocardiography will be performed at every visit. Loops will be recorded and stored as outlined in the echo manual (parasternal long and short axis, 2-, 3- and 4-chamber view, subcostal view). For details, see the attached protocol for Echo-CRF.

3.5 QoL assessment

The standardized and well-established SF-36 questionnaire will be used to evaluate a subjective quality of life of the patients.

4 Safety and risk management

The planned daily dose of spermidine is an approved dietary supplement extracted from spermidine-rich wheat germ (see http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2470&from=EN). Additionally, spermidine is part of a standard healthy diet, present in a wide variety of foods. Therefore, the dose of 4 mg spermidine per day is unlikely to cause any harm to the patients participating in the study. The reduction in blood pressure would be a beneficial therapeutic effect considering the hypertensive baseline. However, a NO/arginine-mediated blood pressure-lowering effect may also cause headaches, dizziness or tachycardia. The study protocol does not interfere with standard baseline therapy. Upon study completion, patients will be invited to participate in the educational program “herz.leben” (ADD link to the webpage!) and, therefore also receive optimal lifestyle modification training.

4.1 Adverse events and reactions

Adverse Events (AE), Adverse Reactions (AR), Unexpected Adverse Reactions (UAR) as well as Serious Adverse Events (SAE), and Serious Adverse Reactions (SAR) will be recorded and reported to the institutional ethics committee as outlined in the ethics approval.

4.2 Study discontinuation

One or more of the following incidences would lead to a discontinuation of the study for a single patient (drop-out):

- Withdrawal of consent
- Intolerable investigational drug-related effects
- Severe violation of the study protocol
- New onset of an exclusion criterion during the treatment period
- Decrease in blood pressure of more than 40 mmHg with symptomatic hypotension

Early discontinuation of the study will be taken into a consideration if the risk-benefit relationship worsens:

- Intolerable investigational drug-related effects in more than 10 patients
- Decrease in blood pressure of more than 40 mmHg with symptomatic hypotension in more than 10 patients

4.3 Potential risks within the study design

All clinical assessments in this trial are standard methods and well established within the Department of Cardiology (MedUniGraz) and its clinical trial section. Thus, the applicant does not expect any methodological risk with respect to the trial. The recruitment of patients into this clinical trial will be based on the cooperation between the applicant and the herz.leben project, in which 120 highly motivated patients per year have been prospectively enrolled since 2007. Therefore, we do not anticipate having problems with recruiting 42 patients from this educational program within 12 months and expect these patients to fully adhere to the study protocol.

Saliva collection by swabbing is a very convenient and non-invasive method for collecting cells in clinical practice. However, saliva samples might not contain enough cells to perform all analyses, including the assessment of autophagic activities. Therefore, whole blood and blood cells (e.g. PBMCs) will be used to detect differences in autophagy, immune cell, and polyamine metabolite profiles. We work under the hypothesis that spermidine induces autophagy in hypertensive patients. If we fail to detect increased autophagy markers upon spermidine supplement, we will use

our data on plasma metabolites (such as L-arginine) and any detected changes in circulatory cell profiles to propose an alternative mode of action (potentially autophagy-independent). If spermidine levels are not increased in whole blood or plasma, our efforts will concentrate on the detection of spermidine-induced changes in polyamine metabolism by analyzing metabolites (i.e. precursors and derivatives of spermidine) in human plasma samples. Moreover, saliva samples offer an additional "tissue"-like sample that will prove the bioavailability of spermidine originating from the administered supplements.

5 Statistical considerations and data analysis

Obtaining repeated measurements from the same patients in response to both treatments has fundamental physiological reasoning (see 3.1) and necessitates completing the study protocol. For this reason and because the trial is designed to measure the treatment efficacy, rather than adherence, patients who drop out before completing the protocol (4 visits) or their baseline treatment regimen changes due to medical reasons will be excluded to maintain a balanced crossover analysis of this trial.

The effect of distinct treatments (spermidine or placebo) on a given parameter (e.g. SBP) will be expressed as a change score from baseline (Δ). The difference between treatment effects will then be evaluated using a mixed linear model, where *Subjects* will be included as a random factor, whereas Treatment (spermidine *vs*. placebo), *Period* (first *vs*. second), and *Sequence* (spermidine-placebo *vs*. placebo-spermidine) will be fixed factors (*i.e.* predictors). Although we expect no carryover effects due to the washout period, including *Period* and *Sequence* in the model will help exclude any confounding effects of (or interaction with) these aspects, especially taking into account that the obtained data will be well-balanced due to the (2x2) study design.

6 Ethical considerations and informed consent

The study shall be conducted in accordance with the ethical principles originating from the *Declaration of Helsinki*[23] and in accordance with *ICH-Good Clinical Practice* (GCP) and applicable regulatory requirements. The study is registered at the local ethics committee with the number 30-468 ex 17/18.

Before randomisation, patients will be comprehensively informed about spermidine and its effects as a compound and the procedures within the trial, but they will be blinded to the treatment sequence (spermidine *vs.* placebo). Participants will be given the informed consent form and given enough time to consider voluntary participation.

7 Data collection and quality assurance, Data management, Monitoring

A unique ID number will be attributed to every enrolled patient. Patients' names will be protected and accessible only to the personnel/the applicant involved in the study. Blood/serum samples will be stored in an anonymous fashion and biologically destroyed 5 years after completion of the study.

On-site monitoring will be conducted by the KKS (Koordinierungszentrum für Klinische Studien), MedUniGraz. Accuracy and completeness of the CRF entries, source documents, and other study-related documents will be checked. After each monitoring visit, a report of any significant findings/facts, deviations, and deficiencies will be communicated to the investigator.

Conflict of interest:

Tobias Eisenberg and Frank Madeo declare a potential financial conflict of interest (equity in TLL). They will not analyze any clinical data and will be blinded throughout the lab analysis after the trial.

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Clinical Trial Synopsis