STATISTICAL ANALYSIS PLAN

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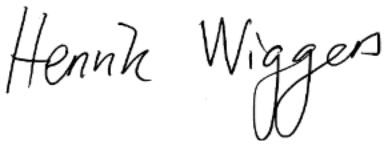

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LIST OF ABBREVIATIONS

16S rRNA: 16S ribosomal ribonucleic acid
ABPM: Ambulatory blood pressure monitoring
ADP: Adenosine diphosphate
AE: Adverse event
ALM: Appendicular lean mass
ALT: Alanine aminotransferase
ANCOVA: Analysis of covariance
ATG5: Autophagy-related protein 5
ATG7: Autophagy-related protein 7
BDNF: Brain-derived neurotrophic factor
BECN1: Beclin 1
BMD: Bone mineral density
BMI: Body mass index
BNIP3: BCL2-interacting protein 3
BNIP3L: BCL2-interacting protein 3-like
BSA: Body surface area
CANTAB: Cambridge Neuropsychological Test Automated Battery
CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration
CMR: Cardiac magnetic resonance imaging
CPET: Cardiopulmonary exercise testing
DMS: Delayed Matching to Sample
DMSPCAD: Delayed Matching to Sample percentage correct across all delays
DNA: Deoxyribonucleic acid
DNM1L: Dynamin 1-like protein
DRP1: Dynamin-related protein 1
DXA: Dual-energy X-ray absorptiometry
E-selectin: Endothelial selectin
ECG: Electrocardiogram
ECV: Extracellular volume fraction
eCRF: Electronic case report form
eGFR: Estimated glomerular filtration rate
eMoCA: Electronic Montreal Cognitive Assessment
ETF: Electron-transferring flavoprotein
FACS: Fluorescence-activated cell sorting
FFQ: Food frequency questionnaire
FUNDC1: FUN14 domain containing protein 1
GDF-15: Growth differentiation factor 15
GPX1: Glutathione peroxidase 1
HbA1c: glycated haemoglobin
HDL: High-density lipoprotein
HeartQoL: HeartQoL health-related quality of life questionnaire
HOMA-IR: Homeostatic Model Assessment for Insulin Resistance
hsCRP: High-sensitivity C-reactive protein
ICAM-1: Intercellular adhesion molecule 1
ICV: Intracellular volume fraction
iECV: Indexed extracellular compartment volume
iICV: Indexed intracellular compartment volume
IL: Interleukin
INR: International normalised ratio
ITT: Intention-to-treat
LC-MS/MS: Liquid chromatography-tandem mass spectrometry

LC3: Microtubule-associated protein 1A/1B-light chain 3
LDL: Low-density lipoprotein
LEAK: Non-phosphorylating LEAK respiration
LV: Left ventricle / left ventricular
LVCO: Left ventricular cardiac output
LVEDV: Left ventricular end-diastolic volume
LVEF: Left ventricular ejection fraction
LVESV: Left ventricular end-systolic volume
LVMi: Left ventricular mass indexed to body surface area
LVSV: Left ventricular stroke volume
LVSVi: Left ventricular stroke volume indexed to body surface area
MAP1LC3A: Microtubule-associated protein 1 light chain 3 alpha
MAP1LC3B: Microtubule-associated protein 1 light chain 3 beta
MCP-1: Monocyte chemoattractant protein 1
MET: Metabolic equivalent
MFN1: Mitofusin 1
MFN2: Mitofusin 2
mgu: Milligravitational units
MIP-1: Macrophage inflammatory protein 1
MRI: Magnetic resonance imaging
NIX: NIP3-like protein X
NT-proBNP: N-terminal pro-B-type natriuretic peptide
OPA1: Optic atrophy 1
PAL: Paired Associates Learning
PALFAMS: Paired Associates Learning first attempt memory score
PALTEA: Paired Associates Learning total errors adjusted
p62: Ubiquitin-binding protein p62 / sequestosome 1
PBMC: Peripheral blood mononuclear cell
PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1 alpha
PINK1: PTEN-induced putative kinase 1
PP: Per-protocol
PPARGC1A: Peroxisome proliferator-activated receptor gamma coactivator 1 alpha
PRDX3: Peroxiredoxin 3
PRKN: Parkin RBR E3 ubiquitin protein ligase
PRM: Pattern Recognition Memory
RCR: Respiratory control ratio
RedCAP: Research Electronic Data Capture
REML: Restricted maximum likelihood
RNA: Ribonucleic acid
RTI: Reaction Time
RTIFMDRT: Reaction Time median five-choice reaction time
RTIFMDMT: Reaction Time median five-choice movement time
RV: Right ventricle / right ventricular
SAE: Serious adverse event
SAP: Statistical analysis plan
SD: Standard deviation
SOD2: Superoxide dismutase 2
SQSTM1: Sequestosome 1
SWM: Spatial Working Memory
SWMBE468: Spatial Working Memory between errors calculated across four-, six- and eight-token trials
SWMS: Spatial Working Memory strategy score calculated across trials with six to eight tokens
TEAE: Treatment-emergent adverse event
TESAE: Treatment-emergent serious adverse event

TFAM: Mitochondrial transcription factor A
TNF-α: Tumor necrosis factor alpha
TSH: Thyroid-stimulating hormone
VAT: Ventilatory anaerobic threshold
VCAM-1: Vascular cell adhesion molecule 1
VCO_2: Carbon dioxide output
VE: Minute ventilation
VE/VCO_2 slope: Ventilatory efficiency slope
VO_2: Oxygen uptake
VRM: Verbal Recognition Memory
WHO-5: World Health Organization-Five Well-Being Index

SAP VERSION HISTORY

Version	Version date	Description
1	26.06.2026	Final version

1 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to provide a comprehensive elaboration of the data handling and analysis to be included in the PolyCAD trial. All decisions regarding analysis strategies as outlined in the SAP will be made prior to the database lock.

2 STUDY HYPOTHESES, OBJECTIVES AND ENDPOINTS

2.1 Overall study organization and primary objective

The overall aim of the trial is to evaluate whether 48 weeks of spermidine supplementation, compared with placebo, affects cardiac remodeling, physical performance, body composition and systemic inflammation in older adults with ischaemic heart disease. To address this aim, the trial is organised into four separate pre-specified efficacy domains. Each domain is based on a distinct hypothesis and includes one primary endpoint with associated secondary and exploratory endpoints as well as a pre-specified sample size estimation. The corresponding objectives and endpoints for each domain are specified below.

Organization	Domain	Hypothesis	Primary endpoint
Main efficacy domain	**Cardiac remodeling**	Spermidine supplementation compared with placebo reduces left ventricular mass and induces beneficial cardiac remodeling	Left ventricular mass (g)
Main efficacy domain	**Physical performance**	Spermidine supplementation compared with placebo improves peak oxygen uptake	Peak VO_2 (ml/kg/min)
Main efficacy domain	**Body composition**	Spermidine supplementation compared with placebo increases skeletal muscle mass	Lean appendicular mass (kg)
Main efficacy domain	**Inflammation**	Spermidine supplementation compared with placebo reduces systemic inflammation	hsCRP (mg/L)
Exploratory domain	**Cognition**	Spermidine supplementation compared with placebo improves cognitive function	Spatial working memory strategy index

Table 1: Overview of the efficacy domains

2.2 Cardiac remodeling domain

2.2.1 Primary objective

To evaluate whether 48 weeks of spermidine supplementation, compared with placebo, affects cardiac remodeling and improves cardiovascular function in older adults with ischaemic heart disease.

2.2.2 Secondary objectives

- To evaluate the effects of spermidine supplementation on vascular function
- To evaluate the effects of spermidine supplementation on ambulatory blood pressure
- To evaluate the effects of spermidine supplementation on circulating biomarkers of vascular function

2.2.3 Primary endpoint

The primary endpoint of the cardiac remodeling domain will be the change from baseline to Week 48 in left ventricular mass (g) assessed by cardiac magnetic resonance imaging (CMR).

2.2.4 Secondary endpoints

- Change from baseline to Week 48 in 24-hour ambulatory systolic blood pressure assessed using the Spacelabs Healthcare 90217A device.
- Change from baseline to Week 48 in 24-hour ambulatory diastolic blood pressure assessed using the Spacelabs Healthcare 90217A device.
- Change from baseline to Week 48 in mean arterial pressure-adjusted pulse wave velocity assessed non-invasively by carotid-femoral applanation tonometry (SphygmoCor®).

2.2.5 Exploratory endpoints

- Change from baseline to Week 48 in circulating biomarkers of cardiac function (GDF-15, NT-proBNP)
- Change from baseline to Week 48 in pulse wave analysis parameters assessed non-invasively by carotid-femoral applanation tonometry (SphygmoCor ®):
    - Aortic end-systolic pressure (mmHg)
    - Aortic augmentation pressure (mmHg)
    - Augmentation index (%)
    - Augmentation index normalised to a heart rate of 75 min^{-1}(%)
    - Ejection duration (ms)
    - Ejection duration (%)
    - Subendocardial Viability Ratio (%)
- Change from baseline to Week 48 in CMR parameters of:
    - Left ventricular end-diastolic volume (LVEDV) (ml)
    - Left ventricular end-systolic volume (LVESV) (ml)
    - Left ventricular stroke volume (LVSV) (ml)
    - Left ventricular stroke volume index (LVSVi) (ml/m^2)
    - Left ventricular ejection fraction (LVEF) (%)
    - Left ventricular cardiac output (LVCO) (L/min)
    - Left ventricular mass indexed to body surface area (LVMi) (g/m^2)
    - Left atrial maximum volume (ml)
    - Left atrial maximum volume index (ml/m^2)
    - Peak global longitudinal strain
    - Extracellular volume fraction (ECV) (%)
    - Indexed extracellular compartment volume (iECV) (ml/m^2)
    - Intracellular volume fraction (ICV) (%)
    - Indexed intracellular compartment volume (iICV) (ml/m^2)

2.3 Physical performance domain

2.3.1 Primary objective

To evaluate whether 48 weeks of spermidine supplementation, compared with placebo, improves physical performance in older adults with ischaemic heart disease.

2.3.2 Secondary objectives

- To evaluate the effects of spermidine supplementation on physical activity

2.3.3 Primary endpoint

- Change from baseline to Week 48 in peak oxygen consumption (ml/min/kg) assessed by cardiopulmonary exercise test (CPET)

2.3.4 Secondary endpoints

- Change from baseline to Week 48 in peak workload during CPET
- Change from baseline to Week 48 in 6-minute walk distance (m)

2.3.5 Exploratory endpoints

- Change from baseline to Week 48 in CPET parameters of:
    - Ventilatory efficiency (VE/VCO_2 slope)
    - Exercise duration during CPET (minutes:seconds)
    - Peak heart rate (min^{-1})
    - O_2-pulse (VO_2 / heart rate)
    - Circulatory power (VO_2 x systolic blood pressure)

  - Rate pressure product (heart rate x systolic blood pressure)
  - VO_2 at ventilatory anaerobic threshold (VAT)
  - Oxygen uptake efficiency slope (ml O_2/min/$\log_{10}$ ventilation (L/min))
  - VO_2-workload slope (ml O_2/min/watt)
- Change from baseline to Week 48 in circulating parameters of:
  - Haemoglobin (mmol/L)
  - Erythrocyte volume fraction
  - Reticulocyte count (x 10^9/L)
- Change from baseline to Week 48 in accelerometry parameters of:
  - Sedentary time (minutes/day)
  - Light physical activity (minutes/day)
  - Moderate physical activity (minutes/day)
  - Moderate to vigorous physical activity (minutes/day)
  - Mean acceleration (milligravitational units (mgu))
  - Intensity gradient
  - Daily step count (steps/day)
  - Sleep time (minutes/night)
  - Peak 5-minute acceleration (mgu)
  - Peak 15-minute acceleration (mgu)
  - Peak 30-minute acceleration (mgu)
  - Peak 60-minute acceleration (mgu)
  - Peak 120-minute acceleration (mgu)

2.4 Body composition domain

2.4.1 Primary objective

To evaluate whether 48 weeks of spermidine supplementation, compared with placebo, improves skeletal muscle mass in older adults with ischaemic heart disease.

2.4.2 Secondary objectives

- To evaluate the effects of spermidine supplementation on body composition
- To evaluate the effects of spermidine supplementation on skeletal muscle strength and function

2.4.3 Primary endpoint

- Change from baseline to Week 48 in appendicular lean mass (kg) assessed by dual-energy X-ray absorptiometry (DXA)

2.4.4 Secondary endpoints

- Change from baseline to Week 48 in isokinetic knee extensor strength (Nm), assessed using a CON-TREX dynamometer.
- Change from baseline to Week 48 in isokinetic knee flexor strength (Nm), assessed using a CON-TREX dynamometer.
- Change from baseline to Week 48 in handgrip strength (kg), assessed using a dynamometer.
- Change from baseline to Week 48 in 30-second sit-to-stand (repetitions).
- Change from baseline to Week 48 in appendicular lean mass (kg) indexed to body mass index and height squared (DXA).
- Change from baseline to Week 48 in total lean body mass (kg) and total lean mass indexed to height squared (DXA).
- Change from baseline to Week 48 in total fat mass (kg) and body fat percentage (%) (DXA).
- Change from baseline to Week 48 in visceral adipose tissue index and visceral adipose tissue mass (g, DXA).

- Change from baseline to Week 48 in thigh muscle volume and mass, assessed by magnetic resonance imaging (MRI).

2.4.5 Exploratory endpoints

- Change from baseline to Week 48 in plasma spermidine concentration (ng/mL), quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS).
- Change from baseline to Week 48 in skeletal muscle spermidine concentration (ng/g), quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS).
- Change from baseline to Week 48 in concentrations of spermine, putrescine, ornithine, arginine, citrulline in skeletal muscle and plasma (ng/g), quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS).
- Change from baseline to Week 48 in proteomic profiles and autophagy-related markers in skeletal muscle and peripheral blood mononuclear cells.
- Change from baseline to Week 48 in mitochondrial respiration parameters:
    - Complex I-supported respiration.
    - Complex I+II-supported respiration.
    - Maximal electron transport system capacity.
    - Fatty acid oxidation-supported respiration (ETF-linked; octanoylcarnitine + malate)
    - Respiratory control ratio (RCR), calculated as Complex I-supported respiration / Complex I-supported leak respiration.
- Change from baseline to Week 48 in muscle quality and characterization outcomes:
    - Peak torque/appendicular lean mass
    - Peak torque/thigh muscle volume (MRI)
    - Intramuscular and intermuscular fat content of thigh (MRI)
    - Skeletal muscle vascularity, morphology, and muscle architecture from vastus lateralis muscle biopsies.
    - Advanced skeletal muscle biopsy measures, including fluorescence-activated cell sorting (FACS).
    - Skeletal muscle cellular composition, assessed by cell sorting.
    - Skeletal muscle satellite cell proliferation and differentiation, assessed by cell number and cell viability assays.
    - Skeletal muscle tissue molecular profiles, assessed by RNA sequencing and by metabolomic and proteomic profiling using mass spectrometry.
    - Skeletal muscle metabolic profiles, assessed by liquid chromatography-high-resolution mass spectrometry.
    - Skeletal muscle fibre type composition, assessed as the ratio of type I, IIa, and IIb fibres by immunohistochemistry.
- Change from baseline to Week 48 in metabolic biomarkers:
    - Insulin resistance, assessed by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR).
    - Free fatty acid concentration, measured from blood samples.
    - Adipose tissue cellular and molecular characteristics, including enzymes involved in lipid storage, cellular composition assessed by fluorescence-activated cell sorting, and downstream DNA/RNA or protein analyses.
- Change from baseline to Week 48 in gut microbiota composition, analysed from stool samples:
    - 16S rRNA analysis and full sequencing for characterisation of bacteria, viruses, bacteriophages, fungi, and parasites.
    - Faecal metabolite profiles, assessed by mass spectrometry-based metabolome analyses.

2.5 Inflammation domain

2.5.1 Primary objective

To evaluate whether 48 weeks of spermidine supplementation, compared with placebo, reduces inflammation in older adults with ischaemic heart disease.

2.5.2 Secondary objectives

- To evaluate the effects of spermidine supplementation on endothelial markers.
- To evaluate the effects of spermidine supplementation on circulating immune cells.

2.5.3 Primary endpoint

- Change from baseline to Week 48 in high-sensitivity C-reactive protein (mg/l).

2.5.4 Secondary endpoints

- Change from baseline to Week 48 in circulating IL-1, IL-6 and TNF-α.

2.5.5 Exploratory endpoints

- Change from baseline to Week 48 in circulating inflammatory markers (IL-10, MCP-1, MIP-1).
- Change from baseline to Week 48 in circulating markers of endothelial function (ICAM-1, E-selectin, VCAM).
- Change from baseline to Week 48 in neutrophil to lymphocyte ratio.
- Change from baseline to Week 48 in white blood cell differential count.

2.6 Exploratory cognition domain

2.6.1 Primary objective

To evaluate whether 48 weeks of spermidine supplementation, compared with placebo, improves cognitive function in older adults with ischaemic heart disease.

2.6.2 Secondary objectives

- To evaluate the effects of spermidine supplementation on health-related quality of life
- To evaluate the effects of spermidine supplementation on mental well-being

2.6.3 Primary endpoint

- Change from baseline to Week 48 in Spatial working memory 6-8 box strategy index (SWMS) assessed by Cambridge Neuropsychological Test Automated Battery (CANTAB)

2.6.4 Secondary endpoints

- Change from baseline to Week 48 in Spatial working memory between errors index in 4, 6 and 8 token trials (SWMBE468)
- Change from baseline to Week 48 in total adjusted errors (PALTEA)

2.6.5 Exploratory endpoints

- Change from baseline to Week 48 in specific domains of cognitive function, assessed by the Cambridge Cognition CANTAB battery:
    - Reaction Time (RTI)
        - Median five-choice reaction time (RTIFMDRT)
        - Median five-choice movement time (RTIFMDMT)
    - Delayed Match to Sample (DMS)
        - Percentage of correct box choices across all delays (DMSPCAD)
    - Paired Associates Learning (PAL)
        - First attempt memory score (PALFAMS)

- Change from baseline to Week 48 in general cognitive function, assessed by the Montreal Cognitive Assessment
- Change from baseline to Week 48 in circulating Brain Derived Neurotrophic Factor (BDNF)
- Change from baseline to Week 48 in WHO-5 score
- Change from baseline to Week 48 in HeartQoL global score
- Change from baseline to Week 48 in HeartQoL physical score
- Change from baseline to Week 48 in HeartQoL emotional score

2.7 Safety Endpoints

Safety will be assessed during the baseline and follow-up study visits as well as during telephone calls 2 weeks and 24 weeks following randomisation. The following

- Number of adverse and serious adverse cardiovascular events
- Number of adverse and serious adverse non-cardiovascular events
- Number of adverse and serious adverse events leading to study drug discontinuation
- Incidence of worsening kidney function, defined as a ≥30% decline in estimated glomerular filtration rate (eGFR) from baseline or progression to eGFR <30 mL/min/1.73 m²
- Incidence of worsening liver function, defined as alanine aminotransferase (ALT) >3 × the sex specific laboratory upper limit of normal

3 STUDY DESIGN

3.1 Summary of Study Design

PolyCAD is a phase II, randomised, double blind, placebo controlled, single centre trial designed to evaluate the cardiometabolic efficacy and safety of spermidine treatment in patients >65 years of age with chronic ischaemic heart disease. Following screening, participants will undergo baseline assessments within 14 days of randomisation (Table 1). Follow-up assessments will be conducted 48 weeks following randomisation within a time window of ±4 weeks. Detailed information on the study design, eligibility criteria, randomisation, blinding, intervention, outcome assessments, and follow-up procedures is provided in the previously published study protocol (1).

Visit		V0 Screening	V1-V3 Baseline studies & Randomisation	T1 Telephone contact	T2 Telephone contact	V4-V6 Follow-up studies
Time (weeks)		-8 to 0	-2 to 0	2 ± 1	24 ± 6	48 ± 4
General						
Informed consent		x				
Medical history		x	x	x	x	x
Clinical examination		x	x			x
Blood samples		x	x			x
Quality of life questionnaire			x			x
Accelerometry			x			x
Cognitive function			x			x
Cardiovascular function						
Cardiac MRI			x			x
Cardiopulmonary exercise test (CPET)			x			x
24h ABPM			x			x
Arterial stiffness			x			x
Echocardiography		x				
Skeletal muscle						
Appendicular lean mass (DXA)		x				x
Muscle strength (dynamometer)			x			x
6-minute walk test			x			x
30 sec. sit to stand test			x			x
Muscle biopsy			x*			x*
Body composition with Dixon MRI			x			x
Metabolism						
Spermine intake (FFQ)			x			
Spermidine concentration in plasma and skeletal muscle biopsies (LC-MS/MS)			x			x
Body composition (DXA)			x			x
Blood samples			x			x
HOMA-IR			x			x

Adipose tissue biopsy			x*			x*
Gut microbiota			x**			x**
Inflammation						
hsCRP			x			x
Inflammation assays			x			x
White blood cells			x			x
Study drug and safety						
-Dispensing, accountability			x	x	x	x
-Compliance, side effects			x	x	x	x
-Concomitant medication		x	x			x
	*Patients can choose to participate in the study without muscle and/or adipose tissue biopsy. **Patients can choose to participate in the study without collecting stool samples.					

Table 2: Study timeline

3.2 Definition of Study Drugs

In Table 3 the study drug (spermidine) and placebo administered in trial are described.

	Active	**Placebo**
Product name	Spermidine	Placebo
Type	Dietary supplement	Dietary supplement
Dose Formulation	Capsule	Capsule
Unit Dose Strength(s)	8 mg (mixed in rice flour)	Matching placebo (rice flour)
Dosage Level(s)	Three capsules daily (24 mg)	Three capsules daily
Route of Administration	Oral	Oral
Use	Interventional study drug	Placebo comparator
Packaging and Labelling	Study product will be provided in identical blinded packaging and labelled in accordance with applicable regulatory requirements.	Study product will be provided in identical blinded packaging and labelled in accordance with applicable regulatory requirements.

Table 3: Definition of study drugs

3.3 Sample Size Considerations

Sample size calculations for each of the four efficacy domains are listed below.

3.3.1 Cardiac remodeling domain

A 10 g change in left ventricular mass is considered clinically relevant. Assuming a standard deviation (SD) of differences of 20 g, a total sample size of 100 patients is needed to detect a 10 g difference of mass. To account for an expected dropout rate of 13%, we plan to include 115 patients for CMR measurements.

3.3.2 Physical performance domain

A change in peak oxygen consumption (peak VO_2) of 6% is considered clinically significant. A recent study in 24 patients at Aarhus University Hospital, with similar patient characteristics as in PolyCAD found a mean peak

VO_2 of approximately 18.3 $mL \cdot kg^{-1} \cdot min^{-1}$ (2). Assuming a SD of differences of 4 mL/kg/min, a total of 156 patients is needed to detect a clinically relevant change in peak VO_2 of 1.85 $mL \cdot kg^{-1} \cdot min^{-1}$. With an expected dropout rate of 13% we plan to enrol 180 patients.

3.3.3 Body composition domain

A between-group difference of 0.5 kg in appendicular lean mass was considered clinically relevant for the sample size calculation. Assuming a 13% dropout rate, approximately 157 of 187 randomised participants are expected to complete follow-up. Based on an assumed standard deviation of 1.0–1.2 kg for repeated DXA-derived measurements and a two-sided alpha level of 0.05, this sample size provides 80% power to detect a between-group difference of approximately 0.45–0.54 kg. For context, a randomised dietary intervention in older men reported a between-group difference in appendicular lean mass of approximately 0.75 kg over 10 weeks (3).

3.3.4 Inflammation domain

Previous trials have demonstrated an approximately 40% reduction in hsCRP in statin treated patients. Assuming a standard deviation for change in hsCRP of 0.6 on the log scale, a two-sided significance level of 0.05, and a dropout rate of 13%, 160 patients will need to be enrolled to provide 80% power to detect a 25% reduction in hsCRP.

3.4 Efficacy Assessments

3.4.1 CMR Assessments

CMR will be performed at baseline prior to randomisation and at follow-up prior to study completion. Scans will be performed using a 1.5-T scanner (Magnetom Sola, Siemens Healthineers, Erlangen, Germany) with a 32-channel cardiac coil and an 18-channel body coil. Cardiac gating will be performed using a three-lead vector electrocardiogram. Analysis will be performed by one blinded investigator prior to data lock. CMR scans will be analysed in CVI 42 (Circle Cardiovascular Imaging, version 6.0.3, Calgary, Alberta, Canada).
For variables indexed to body surface area, the DuBois formula will be used to calculate body surface area based on height and weight:

$$BSA(m^2) = 0.007184 \times (weight(kg)^{0.425} \times height(cm)^{0.725})$$

For all body surface area-indexed parameters, the body surface area calculated from the participant's baseline weight and height will be used for both the baseline and follow-up assessments.

- CMR parameters:
  - Left ventricular mass (g)
  - Left ventricular mass index (g/m^2)
  - Left ventricular end-diastolic volume (ml)
  - Left ventricular end-systolic volume (ml)
  - Left ventricular stroke volume (ml)
  - Left ventricular stroke volume index (ml/m^2)
  - Left ventricular ejection fraction (%)
  - Left ventricular global peak longitudinal strain
  - Left ventricular cardiac output (L/min)
  - Left ventricular cardiac index ($L/min/m^2$)
  - Global native T1 time (ms)
  - Global myocardial T1 time post contrast (ms)
  - Global extracellular volume fraction (ECV) (%)
  - Global indexed extracellular compartment volume
  - Global intracellular volume fraction (ICV) (%)
  - Global indexed intracellular compartment volume

- Left atrial maximum volume (ml)
- Left atrial maximum volume index (ml/m^2)
- Left atrial end diastolic volume (ml)
- Left atrial end systolic volume (ml)

- RV parameters:
  - Right ventricular end diastolic volume (ml)
  - Right ventricular end systolic volume (ml)
  - Right ventricular stroke volume (ml)
  - Right ventricular stroke volume index (ml/m^2)
  - Right ventricular ejection fraction (%)
- Calculated CMR parameters:
  - ECV:
    - ECV%= (ΔR1 myocardium) / (ΔR1 blood pool) x (1 – erythrocyte volume fraction)
    - ΔR1: the difference in relaxation rates (1/T1) pre- and post-gadolinium contrast injection
  - ICV:
    - ICV%=100%-ECV
  - iECV
    - iECV ml/m^2=ECV x (LVMi/1.05)
  - iICV:
    - iICV ml/m^2=ICV x (LVMi/1.05)

3.4.2 CPET Assessments

Patients will undergo CPET (JAEGER Vyntus CPX, Vyaire Medical, IL, USA) with gas-exchange analysis following a standardized ramp protocol aimed at 8-12 minutes of exercise. The specific ramp protocol is chosen at baseline based on fitness level, and participants will undergo the same ramp protocol at baseline and follow-up visits. The following CPET parameters are estimated:

- Oxygen uptake (ml/min) at rest and at peak exercise
- Oxygen uptake indexed to bodyweight (ml/kg/min) at rest and at peak exercise
- Metabolic equivalents (METs) at rest and peak exercise
- Systolic and diastolic blood pressure at rest and peak exercise (mmHg)
- % of predicted peak oxygen uptake
- Respiratory exchange ratio at rest and at peak exercise
- Oxygen pulse (ml O_2/heartbeat) at rest and at peak exercise
- Heart rate (min^{-1}) at rest and at peak exercise
- Heart rate reserve (%) at rest and at peak exercise
- Ventilatory efficiency (VE/VCO_2 slope)
- Oxygen uptake efficiency slope (ml O_2/min/$\log_{10}$ ventilation(L/min))
- Peak workload (watts)
- Exercise duration (minutes)
- Oxygen uptake at anaerobic threshold (ml/min)
- VO_2 workload slope (mlO_2/min/watt)

3.4.3 Blood samples

Blood samples will be collected in the fasting state at baseline prior to randomisation and at the final visit.
The following list of blood samples will be acquired at the baseline and follow-up visits and values will be immediately available:

- Creatine kinase (U/L)
- Total cholesterol (mmol/L)
- HDL cholesterol (mmol/L)
- LDL cholesterol (mmol/L)

- Triglyceride (mmol/L)
- Alanine aminotransferase (U/L)
- Alkaline phosphatase (U/L)
- Glucose (mmol/L)
- Haemoglobin A1c (mmol/mol)
- TSH (IU/L)
- 25-hydroxyvitamin (nmol/L)
- Potassium (mmol/L)
- Sodium (mmol/L)
- Calcium (mmol/L)
- Ionised calcium (mmol/L)
- Magnesium (mmol/L)
- Albumin (g/L)
- Creatinine (µmol/L)
- Urea (mmol/L)
- eGFR (ml/min/1.73 m^2)
- High-sensitivity CRP (mg/L)
- Total leukocytes ($x10^9$/L)
- Neutrophils ($x10^9$/L)
- Lymphocytes ($x10^9$/L)
- Monocytes ($x10^9$/L)
- Eosinophils ($x10^9$/L)
- Basophils ($x10^9$/L)
- Haemoglobin (mmol/L)
- Erythrocyte volume fraction
- Reticulocytes ($x10^9$/L)
- Platelets ($x10^9$/L)
- Iron (µmol/L)
- Transferrin (µmol/L)
- Ferritin (µg/L)
- Vitamin B12 (pmol/L)
- International Normalised Ratio (INR)
- Urate (mmol/L)

After blood sampling, plasma and serum will be separated and stored at -150°C until analysis. Cytokines, interleukins, and organ-specific biomarkers will be measured in a single analytical batch following collection of the final follow-up sample. For hsCRP, the lower detection limit of the assay used is 0.2 mg/L. Values below this threshold will be imputed as 0.1 mg/L. Estimated glomerular filtration rate will be calculated using the 2009 CKD-EPI creatinine equation, based on plasma creatinine, age and sex.

Biomarkers analysed in a single analytical batch:

- MCP-1
- MIP-1
- IL-1
- IL-6
- IL-10
- TNF- α
- ICAM-1
- E-Selectin
- VCAM
- GDF-15

- Insulin
- BDNF
- NT-proBNP

3.4.4 Arterial stiffness

Carotid-femoral pulse wave velocity will be estimated using applanation tonometry (SphygmoCor®). The following parameters will be acquired:

- Pulse wave velocity (m/s)
- Pulse transit time (ms)
- Aortic systolic blood pressure (mmHg)
- Aortic diastolic blood pressure (mmHg)
- Aortic pulse pressure (mmHg)
- Aortic end systolic pressure (mmHg)
- Aortic augmentation pressure (mmHg)
- Augmentation index (%)
- Augmentation index normalised to a heart rate of 75 min^{-1}(%)
- Ejection duration (ms)
- Ejection duration (%)
- Subendocardial Viability Ratio (%)

3.4.5 Muscle strength and function assessments

Muscle strength and physical function outcomes will include isokinetic knee extensor strength (Nm), isokinetic knee flexor strength (Nm), handgrip strength (kg), 6-minute walk distance (m), and 30-second sit-to-stand performance, assessed as the number of completed repetitions. Maximal unilateral knee extensor and flexor strength will be assessed using an isokinetic dynamometer (CON-TREX; Physiomed, Schnaittach, Germany) during maximal voluntary contractions of the dominant leg. Peak torque will be recorded in absolute terms (Nm) and normalised to body mass ($Nm \cdot kg^{-1}$), with the highest value from three contractions used for analysis. Handgrip strength will be assessed using a handheld dynamometer (Charder Medical, Taichung City, Taiwan), with the highest value from three attempts with the dominant hand used for analysis. Submaximal exercise capacity will be assessed using the 6-minute walk test, from which 6-minute walk distance will be derived. Lower-limb functional performance will be assessed using the 30-second sit-to-stand test, recorded as the number of complete rises performed within 30 seconds.

3.4.6 Physical activity

Between screening and randomisation, a triaxial wrist-worn accelerometer (Axivity AX3, Newcastle upon Tyne, United Kingdom) will assess 14-day physical activity. Accelerometry will be repeated prior to the final visit. The following list of accelerometry parameters will be assessed:

- Sedentary time (minutes/day)
- Light physical activity (minutes/day)
- Moderate activity (minutes/day)
- Moderate to vigorous physical activity (minutes/day)
- Vigorous physical activity (minutes/day)
- Mean acceleration (mgu)
- Intensity gradient
- Daily step count (steps/day)
- Sleep time (minutes/night)
- Peak 5-minute acceleration (mgu)
- Peak 15-minute acceleration (mgu)
- Peak 30-minute acceleration (mgu)
- Peak 60-minute acceleration(mgu)
- Peak 120-minute acceleration (mgu)

3.4.7 Patient reported outcomes (HeartQoL)

The Health-related quality-of-life questionnaire (HeartQoL) is a disease-specific measure of health-related quality of life for patients with ischaemic heart disease. It comprises 14 items, including a 10-item physical subscale and a 4-item emotional subscale. Each item is scored on a 4-point Likert scale from 0 to 3, with higher scores indicating better health-related quality of life. HeartQoL scores will be calculated as the mean of the scored items for the global score and, where relevant, for the physical and emotional subscale scores. Accordingly, all HeartQoL scores range from 0 to 3.

3.4.8 Patient reported outcomes (WHO-5)

The World Health Organization-Five Well-Being Index (WHO-5) is a short self-reported measure of current mental well-being comprising 5 items referring to the preceding 2 weeks. Each item is scored from 0 to 5, with higher scores indicating better well-being. The raw score is calculated as the sum of the five item scores and therefore ranges from 0 to 25. To obtain the standard WHO-5 score, the raw score will be multiplied by 4, yielding a scale from 0 to 100, where higher scores indicate better well-being.

3.4.9 Electronic Montreal Cognitive Assessment (eMoCA)

The electronic Montreal Cognitive Assessment (eMoCA) is a screening instrument for general cognitive function. It will be scored according to standard MoCA procedures and yields a total score ranging from 0 to 30, with higher scores indicating better cognitive performance. Education adjustment will be performed according to the manual, as educational level with ≤12 years of education yields one point in the overall score.

3.4.10 Cambridge Neuropsychological Test Automated Battery (CANTAB)

CANTAB is a computerised cognitive test battery that provides test-specific outcome measures rather than a single overall total score. Tests will be administered and scored using the Cambridge Cognition platform. Key CANTAB parameters, selected in accordance with the CANTAB user guidance, will be obtained within the following cognitive domains:

- Reaction Time (RTI)
  - Median five-choice reaction time (RTIFMDRT)
  - Median five-choice movement time (RTIFMDMT)
- Spatial Working Memory (SWM)
  - Spatial working memory 6-8 box strategy index (SWMS)
  - Spatial working memory between errors index in 4, 6 and 8 token trials (SWMBE468)
- Delayed Match to Sample (DMS)
  - Percentage of correct box choices across all delays (DMSPCAD)
- Paired Associates Learning (PAL)
  - Total adjusted errors (PALTEA)
  - First attempt memory score (PALFAMS)

3.4.11 DXA Assessments

Appendicular lean mass will be assessed using DXA (Hologic Inc., Marlborough, MA, USA) following an overnight fast of at least 12 hours. All scans will be performed on the same device by three trained operators according to standardised protocols to ensure consistency and reproducibility. The following DXA parameters are estimated:

- Appendicular lean mass (ALM; kg)
- ALM indexed to body mass index (ALM/BMI)
- ALM indexed to height squared (ALM/height^2)
- Total lean body mass (kg)
- Total lean body mass indexed to height squared
- Total fat mass (kg)
- Body fat percentage (%)
- Visceral adipose tissue mass (g)

- Visceral adipose tissue index
- Bone mineral density (BMD; g/cm²)
- Total body bone mineral density

3.4.12 Thigh MRI Assessments

Thigh muscle volume and fat content will be assessed by magnetic resonance imaging (MRI) using Dixon T1-weighted water and fat images with semi-automated segmentation and three-dimensional reconstruction. Muscle volume will be derived from segmented thigh muscle compartments, and intramuscular fat content will be estimated from Dixon-derived fat-fraction images. For clinical interpretation, muscle mass will be estimated from muscle volume assuming a muscle density of 1.06 $g \cdot cm^{-3}$. MRI thigh scans deemed invalid due to technical errors, data loss during transfer, or poor image quality will be treated as missing.

3.4.13 Skeletal Muscle Biopsy Assessments

Skeletal muscle biopsy analyses will be performed on vastus lateralis samples collected at baseline and Week 48 in a fasted state. Assessments will include skeletal muscle vascularity, morphology, architecture, cellular composition, satellite cell characteristics, fibre type composition, and molecular and metabolic profiles. Cellular composition will be assessed using fluorescence-activated cell sorting and related cell-sorting methods. Cell number, viability, proliferation, and differentiation will be assessed. Fibre type composition will be assessed by immunohistochemistry and reported as the relative proportions of type I, type IIa, and type IIb fibres. Molecular and metabolic profiling will include RNA sequencing, mass spectrometry-based proteomics and metabolomics, and liquid chromatography–high-resolution mass spectrometry. Invalid samples, insufficient tissue material, failed assays, or samples not meeting predefined quality-control criteria will be treated as missing.

3.4.14 Mitochondrial Assessments

Mitochondrial respiratory capacity will be assessed using high-resolution respirometry (Oroboros O2k, Oroboros Instruments, Innsbruck, Austria) in permeabilised skeletal muscle fibres following substrate–uncoupler–inhibitor titration protocols. For carbohydrate-supported respiration, glutamate and malate will be added to assess Complex I-linked LEAK respiration in the absence of ADP. ADP will then be added to determine Complex I-supported oxidative phosphorylation capacity, followed by succinate to assess combined Complex I+II-supported oxidative phosphorylation capacity. Maximal electron transfer system capacity will be assessed by stepwise titration of carbonyl cyanide m-chlorophenyl hydrazone. Rotenone will subsequently be added to inhibit Complex I, and antimycin A will be added to determine residual oxygen consumption. For fatty acid-supported respiration, malate and octanoylcarnitine will be added to assess fatty acid-supported LEAK respiration, followed by ADP to determine fatty acid-supported oxidative phosphorylation capacity. Cytochrome c will be added to assess outer mitochondrial membrane integrity. Respiratory fluxes will be corrected for residual oxygen consumption and normalised to tissue wet weight. Citrate synthase activity will be measured as a marker of mitochondrial content in baseline samples, and respiratory capacities will additionally be normalised to citrate synthase activity in secondary analyses.

3.4.15 Polyamine and Amino Acid Assessments

Polyamines and amino acids will be quantified in plasma and skeletal muscle tissue using targeted liquid chromatography–tandem mass spectrometry (LC-MS/MS). The analyses will include spermidine, spermine, putrescine, and amino acids, with stable isotope-labelled internal standards used for each analyte. Plasma samples and skeletal muscle homogenates will be extracted using methanol-based sample preparation before LC-MS/MS analysis. Analyses will be performed using ultra-performance liquid chromatography coupled to a triple quadrupole mass spectrometer with positive electrospray ionisation and multiple reaction monitoring. Quantification will be based on matrix-matched calibration curves generated from reference compounds and isotope-labelled internal standards. The method has been validated for repeatability, within-laboratory precision, trueness, and analyte stability under relevant storage and processing conditions. Polyamine concentrations will be reported for plasma and skeletal muscle tissue, and the spermine/spermidine ratio will be calculated as a marker of polyamine balance. Key parameters:

- Plasma spermidine

- Plasma spermine
- Plasma putrescine
- Skeletal muscle spermidine
- Skeletal muscle spermine
- Skeletal muscle putrescine
- Plasma polyamine ratios
- Skeletal muscle polyamine ratios
- Plasma and skeletal muscle amino acid concentrations

3.4.16 Proteomic analysis

Untargeted mass spectrometry-based proteomics will be performed in collaboration with the Dengjel group at the University of Fribourg, Switzerland. In addition to global proteome-wide analyses, predefined proteins of interest related to autophagy, mitophagy, mitochondrial quality control, oxidative phosphorylation, and oxidative stress will be evaluated when detected and reliably quantified. Proteins of interest will include autophagy-related markers LC3/MAP1LC3A/B, SQSTM1/p62, BECN1/Beclin-1, ATG5, and ATG7; mitophagy-related markers PINK1, PRKN/Parkin, BNIP3, BNIP3L/NIX, and FUNDC1; mitochondrial biogenesis markers PPARGC1A/PGC-1α and TFAM; mitochondrial dynamics proteins MFN1, MFN2, OPA1, and DNM1L/DRP1; oxidative phosphorylation proteins from mitochondrial respiratory chain complexes I–V; and oxidative stress-related proteins including SOD2, GPX1, and PRDX3.
Proteins of primary autophagy-related analysis will include:

UniprotID	GeneID
O00116	AGPS
Q9C0C7	AMBRA1
Q9P2R3	ANKFY1
Q6DD88	ATL3
P25705	ATP5F1A
Q9BXK5	BCL2L13
O60238	BNIP3L
Q12983	BNIP3
Q9P1Z2	CALCOCO1
Q13137	CALCOCO2
P27797	CALR
P27824	CANX
Q9ULG6	CCPG1
Q8N884	CGAS
P10809	HSPD1
O75390	CS
Q96JB5	CDK5RAP3
P13073	COX4I1
P31327	CPS1
P14625	HSP90B1
Q14318	FKBP8
Q96RU3	FNBP1
Q8IVP5	FUNDC1
Q10471	GALNT2
Q9H0R8	GABARAPL1
P60520	GABARAPL2
Q8TBA6	GOLGA5
Q14789	GOLGB1
Q9H4A6	GOLPH3
Q9H8Y8	GORASP2
Q86UP2	KTN1

P11279	LAMP1
P13473	LAMP2
P20700	LMNB1
Q03252	LMNB2
O75074	LRP3
Q9H492	MAP1LC3A
Q9GZQ8	MAP1LC3B
Q14596	NBR1
Q13772	NCOA4
Q86UT6	NLRX1
Q96CV9	OPTN
P30101	PDIA3
O43933	PEX1
Q92968	PEX13
Q99623	PHB2
Q99541	PLIN2
P51148	RAB5C
P51149	RAB7A
Q9H6L5	RETREG1
Q8NC44	RETREG2
Q86VR2	RETREG3
O95197	RTN3
Q99442	SEC62
Q13596	SNX1
O60749	SNX2
Q8N0X7	SPART
Q13501	SQSTM1
O95210	STBD1
O15400	STX7
Q86VP1	TAX1BP1
Q5BJD5	TMEM41B
Q9H0E2	TOLLIP
Q9Y6I9	TEX264
Q96QK1	VPS35
Q9UN37	VPS4A
Q9GZM5	YIPF3
Q9BSR8	YIPF4

3.4.17 Metabolic biomarker assessments

Metabolic biomarkers will include insulin resistance, free fatty acid concentrations, visceral adipose tissue, and adipose tissue cellular and molecular characteristics. Insulin resistance will be assessed using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), calculated from fasting glucose and fasting insulin concentrations. Free fatty acid concentrations will be measured in blood samples collected at baseline and Week 48. Visceral adipose tissue mass and visceral adipose tissue index will be assessed using DXA. Adipose tissue biopsies will be used to assess cellular and molecular characteristics of adipose tissue, including enzymes involved in lipid storage, cellular composition assessed by fluorescence-activated cell sorting, and downstream DNA-, RNA-, or protein-based analyses. Invalid or insufficient biopsy material, technical errors, or failed laboratory analyses will be treated as missing. Parameters of interest:

- HOMA-IR
- Fasting glucose concentration
- Fasting insulin concentration
- Free fatty acid concentration

- Adipose tissue lipid storage-related enzyme markers
- Adipose tissue cellular composition
- Adipose tissue DNA-based markers
- Adipose tissue RNA-based markers
- Adipose tissue protein-based markers

3.4.18 Gut microbiota and faecal metabolomics assessments

Gut microbiota composition and faecal metabolite profiles will be assessed from stool samples collected at baseline and Week 48. Microbial composition will be analysed using 16S rRNA sequencing and shotgun metagenomic sequencing, where available, to characterise bacterial composition and broader microbial communities, including viruses, bacteriophages, fungi, and parasites. Faecal metabolite profiles will be assessed using mass spectrometry-based metabolomics. Invalid samples, insufficient sample material, failed sequencing, failed metabolomics analyses, or samples not meeting predefined quality-control criteria will be treated as missing.

- Gut microbiota composition
- Bacterial composition assessed by 16S rRNA sequencing
- Shotgun metagenomic microbial community profiles
- Viral community profiles
- Bacteriophage profiles
- Fungal community profiles
- Parasitic community profiles
- Faecal metabolite profiles

4 STUDY POPULATION

4.1 Subjects Disposition

The disposition of the study population will be described using a consort diagram. The CONSORT diagram will summarize the number of invited patients, screened patients, screen failures, randomised patients and patients included in the analysis.
Screen failures will be listed and reasons for screen failure will be summarized. For the patients who discontinued from the study prior to the follow-up visit, reasons for discontinuation will be summarized. For patients who are not included in the final analysis, reasons for exclusion from analysis will be summarized.

4.2 Demographic and Baseline Characteristics

Baseline characteristics including demographics, age, sex, height, weight, randomisation stratification, baseline medication use (see 4.4 Baseline Medications Use) and comorbidities will be summarized for all randomised patients included in the intention-to-treat analysis population. No hypothesis testing of baseline characteristics will be performed. Data will be presented using descriptive statistics as means ± standard deviations, medians with interquartile ranges and proportions where appropriate.

4.3 Baseline Definitions

Baseline is defined as the last available measurement taken prior to administration of first study related dose.
Baseline age will be calculated at screening based on the following: baseline age = screening date – birth date.
Change from baseline will be calculated as follow-up value – baseline value.
Relative change from baseline will be calculated as ((follow-up value – baseline value)/ baseline value X 100)

4.4 Baseline Medications Use

Baseline medication use will be defined as concomitant medical therapy started prior to first dose of intervention. This will be recorded at the screening visit. If any medication changes occured between screening and randomisation an updated medication list will be uploaded in the eCRF at the randomisation visit and the last recorded medication list prior to first dose of intervention will be used for baseline medication.

4.5 Analysis Populations

4.5.1 All screened patients

All patients who attended screening and signed the informed consent form. Screen failures will be defined as participants who did not progress to baseline assessments and randomisation. The following reasons for screen failures were collected: not meeting eligibility criteria, subject decision or serious adverse events during the screening period prior to randomisation. The reasons for screen failure will be summarized.

4.5.2 Intention-to-treat analysis population

The intention-to-treat population will include all randomised participants, analysed according to their randomised treatment allocation. As criteria for withdrawal from the study were prespecified in the published protocol and listed in 4.5.5 Withdrawal from the study, patients meeting these criteria will not be included in the intention-to-treat population (1).

4.5.3 Per-protocol analysis population

The per-protocol population will include all randomised participants with adherence to ≥80% of the prescribed study intervention.

4.5.4 Safety analysis population

All randomised patients who received at least one dose of study treatment.

4.5.5 Withdrawal from the study

Withdrawal from the trial will occur under the following conditions:

- Voluntary withdrawal of consent: Participants may withdraw from the study at any time, without providing a reason and without any impact on their current or future medical care
- Refusal to attend follow-up visits: Failure to respond to multiple contact attempts, defined operationally as no response to three phone calls and text messages over a 2-week period. All contact attempts will be documented in the electronic case report form (eCRF)
- Withdrawal by investigators for any of the following predefined reasons:
    - Development of a new, clinically significant comorbidity (e.g. malignancy or major cardiovascular events).
    - Serious adverse events (SAEs) preventing the participants from completing the subsequent study, as determined by the investigator's judgement.
    - Major protocol violations impacting participant safety or data integrity. Any other safety-related concerns deemed critical by the investigator.
    - False enrolment for not meeting eligibility criteria.

A summary of patients who withdrew from the study and did not participate in follow-up will be provided.

5 EFFICACY ANALYSIS

5.1 Final Analyses

The final analysis will be performed after completion of follow-up, resolution of data queries, and database lock. Unblinding for the confirmatory efficacy analyses will occur only after the SAP has been finalised and approved. No interim analysis was planned in this study.

5.2 General Summary Table and Individual Subject Data Listing Considerations

Continuous variables will generally be summarised using number of observations, mean, standard deviation, median, quartiles, minimum, and maximum. Categorical variables will be summarised using counts and percentages. Listings will be produced where required for protocol deviations, adverse events, discontinuations, and important endpoint reviews.

5.3 Data Management

Data will be entered into Research Electronic Data Capture (REDCap), hosted at Aarhus University. Data will be reviewed and cleaned prior to data lock and statistical analysis.

5.4 Statement of the Null and Alternate Hypotheses

The null hypotheses for the four main efficacy domains are:

- **Cardiac remodeling domain:**
  - The treatment difference (spermidine vs. placebo) of mean change in left ventricular mass (g) from baseline to Week 48 is 0. The alternative hypothesis is that the treatment difference is <0 (favours spermidine). Only the direction favouring spermidine will be considered success.
- **Physical performance domain:**
  - The treatment difference (spermidine vs. placebo) of mean change in peak VO_2 (ml/min/kg) from baseline to Week 48 is 0. The alternative hypothesis is that the treatment difference is >0 (favours spermidine). Only the direction favouring spermidine will be considered success.
- **Body composition domain:**
  - The treatment difference (spermidine vs. placebo) of mean change in appendicular lean mass (kg) from baseline to Week 48 is 0. The alternative hypothesis is that the treatment difference is >0 (favours spermidine). Only the direction favouring spermidine will be considered success.
- **Inflammation domain:**
  - The treatment difference (spermidine vs. placebo) of mean change in high-sensitivity C-reactive protein (mg/l) from baseline to Week 48 is 0. The alternative hypothesis is that the treatment difference is <0 (favours spermidine). Only the direction favouring spermidine will be considered success.

5.5 Primary Efficacy Analysis

In each of the four main efficacy domains, the primary endpoint will be analysed using an analysis of covariance (ANCOVA) model. For each primary endpoint, the Week 48 variable will be included as the dependent variable, treatment group (spermidine or placebo) will be included as a fixed effect and the corresponding baseline value as a covariate. The placebo group will be used as the reference group. The treatment effect will be estimated as the adjusted between-group difference in the Week 48 value, with corresponding 95% confidence interval and a two-sided p-value of 0.05.

Model assumptions will be assessed by graphical examination of residuals versus fitted values and QQ-plots of the residuals. Log transformation will be employed if model assumptions are not met. If model assumptions are substantially violated non-parametric analysis will be performed.

The primary analysis will be performed on the intention-to-treat population with available baseline and follow-up values for the respective endpoint.

For the Inflammation domain, hsCRP will be analysed using ANCOVA on the natural logarithmic scale, with log-transformed Week 48 hsCRP as the dependent variable, treatment group as a fixed effect, and log-transformed baseline hsCRP as a covariate. The treatment effect will be back-transformed and reported as the adjusted geometric mean ratio between treatment groups, with corresponding 95% confidence interval and two-sided p-value of 0.05. The relative difference will be reported.

5.6 Testing strategy and Multiplicity

The trial evaluates four separate pre-specified efficacy domains, each represented by a corresponding primary endpoint and addressing a distinct hypothesis. No formal adjustment for multiplicity will be applied across the four primary endpoints, and no overall trial-level success criterion will be defined. Accordingly, interpretation will consider the number of hypotheses tested, the magnitude and precision of the estimated treatment effects, and the consistency of findings across related endpoints within a specific efficacy domain.

No formal adjustment for multiplicity will be applied to secondary, exploratory, subgroup or subdomain (see 2.6 Exploratory cognition domain) analyses. Results from these analyses will be interpreted cautiously and considered hypothesis-generating.

5.7 Missing data

For each efficacy domain, the primary analysis will be based on participants with available baseline and Week 48 data for the corresponding endpoint. The number, proportion and reasons for missing will be summarized by treatment group for each primary endpoint. Baseline characteristics will be summarized separately for participants with and without available Week 48 data.

5.8 Data validity for the primary endpoints

5.8.1 CMR

CMR scan sequences deemed to be invalid due to technical errors and data loss during data transfer or poor image quality will be treated as missing.

5.8.2 CPET

CPET data will be assessed by the investigators and deemed invalid and treated as missing data if any of the following are true:

- Technical failure during the procedure (suspected air leak or miscalibration of gas exchange etc.)
- Major CPET process violation that could impact CPET maximal effort variables
- Transient non-cardiac issues that could preclude accurate conduct of testing or acquisition of data

5.8.3 DXA

Quality control includes visual inspection of all scans and exclusion or repeat analysis of scans with movement artefacts or technical errors.

5.8.4 hsCRP

At baseline and follow-up visits, symptoms and signs suggestive of acute infection or another acute inflammatory condition will be recorded. Before database lock, hsCRP values will be reviewed blinded to treatment allocation. A hsCRP measurement will be considered non-evaluable for the primary analysis if there is clear evidence of an acute intercurrent inflammatory condition likely to materially affect hsCRP, such as investigator-documented infection, body temperature ≥38.0°C, initiation of antimicrobial therapy within ±7 days

of sampling, or another acute inflammatory event requiring medical assessment. Repeat sampling should be attempted after approximately 2 weeks, when clinically stable, and the repeated value should be used for the main analysis. If repeated sampling is not possible, the value at the corresponding visit will be treated as missing.

5.9 Sensitivity Analyses of the Primary Efficacy Endpoints

Sensitivity analysis of the primary outcomes will be performed as defined below:

- A per protocol analysis excluding patients with compliance <80%
- An analysis of each of the primary endpoints using a linear mixed effects model including patients with outcomes missing at either baseline or follow-up. The model will use treatment group (spermidine or placebo) and visit (baseline or follow-up) as fixed effects and patient as random intercept. The treatment effect will be estimated from the interaction between treatment group and visit.
- If substantial outliers are present in the data a sensitivity analysis excluding these will be performed.
- An analysis excluding the patients that started new antihypertensive treatment during the study.
- An analysis excluding participants who initiated weight-loss medication (e.g. glucagon-like peptide-1 receptor agonists) during the study period.

5.10 Subgroup Analyses

Subgroup analyses will be conducted by extending the primary model to include the subgroup variable and a treatment-by-subgroup interaction term. Interaction p-values will be reported. Subgroup-specific treatment effects and 95% confidence intervals will be presented where the number of participants is sufficient to support reliable model estimation. These analyses will be considered exploratory, and no adjustment for multiplicity will be applied.
The following baseline characteristics will be used to perform subgroup analysis in all four primary endpoints:

- Age (≥median age versus < median age)
- Sex (male versus female)
- Diabetes status (diabetes vs no diabetes).
- BMI (BMI ≥30 kg/m² versus <30 kg/m²)

For each of the four efficacy domains the following baseline values will be used to perform subgroup analysis specific to the corresponding primary endpoint.

5.10.1 The cardiac remodeling domain

- Left ventricular hypertrophy on CMR (>59 g/m^2 for males and >48 g/m^2 for females versus ≤59 g/m^2 for males and ≤48 g/m^2 for females)(4)
- Blood pressure control (24-hour ambulatory blood pressure) (systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥90 mmHg versus systolic blood pressure <130 mmHg and diastolic blood pressure <90 mmHg)
- Myocardial infarction (prior myocardial infarction versus no prior myocardial infarction)

5.10.2 The physical performance domain

- Beta blocker use (beta blocker versus no beta blocker)
- Physical activity from 14-day accelerometry (≥median average acceleration versus <median average acceleration (mgu))

5.10.3 The body composition domain

- Appendicular lean mass indexed to height squared (≤7.0 kg/m² for men and ≤5.5 kg/m² for women versus >7.0 kg/m² for men and >5.5 kg/m² for women)
- Physical activity from 14-day accelerometry (≥median average acceleration versus <median average acceleration (mgu))

5.10.4 The inflammation domain

- hsCRP (≥2 mg/l versus <2 mg/l)

5.11 Analysis of the Secondary and Exploratory Efficacy Endpoints

Treatment effects for secondary and exploratory endpoints will be analysed using an ANCOVA model with treatment (spermidine or placebo) as a fixed effect and the corresponding baseline value as a covariate.

Model assumptions will be assessed by graphical examination of QQ-plots of the residuals, and log transformation will be employed if model assumptions are not met. If model assumptions are substantially violated non-parametric analysis will be performed.

5.11.1 Analysis of patient reported outcomes

The HeartQoL and WHO-5 summary scores will only be calculated if all individual item scores are available. For the HeartQoL global score and the WHO-5 summary score, the treatment effect will be analysed using an ANCOVA model of the treatment effect including the global scores (HeartQoL or WHO-5) at Week 48 as the dependent variable, treatment (spermidine or placebo) as a fixed effect and the corresponding baseline score as a covariate. If model assumptions are severely violated, data will be log-transformed and if assumptions are not met, descriptive summaries including medians and interquartile ranges will be reported.

Ceiling effects have previously been reported for the HeartQoL instrument, particularly the emotional score (5–7). The score of 3 indicates absence of reported limitations or concerns captured by the corresponding scale.
In the presence of a substantial ceiling effect, each HeartQoL scale will be analysed in an exploratory dichotomized analysis, categorizing participants as having minimal reported limitations, defined as a score >2.9, or reported limitations, defined as a score ≤2.9. For each scale, the probability of attaining the maximum score at follow-up will be evaluated using logistic regression with treatment group as a fixed effect and the corresponding baseline HeartQoL score as a covariate. Results will be reported as adjusted odds ratios with corresponding 95% confidence intervals. If the distribution of participants across categories is too imbalanced to support reliable model estimation, descriptive analyses only will be presented. In this case, the proportions of participants within each category at baseline and follow-up will be summarized by treatment group.

5.11.2 Analysis of variables from the CANTAB and MoCA tests

Change scores from baseline to Week 48 in CANTAB variables and MoCA total score will be assessed using an ANCOVA model with treatment (spermidine or placebo) as a fixed effect and the corresponding baseline value as a covariate. Model assumptions will be assessed by graphical examination of QQ-plots of the residuals, and log transformation will be employed if model assumptions are not met. If model assumptions are substantially violated non-parametric analysis will be performed.

5.12 Prespecified exploratory pooled analysis

Data from the PolyCAD trial will be pooled with data from the SMARTEST trial (NCT04405388). The main objective of the pooled analysis is to estimate the effect of spermidine supplementation compared with placebo on cardiovascular and inflammatory outcomes from baseline to the final scheduled follow-up. Given the differences in study design, two complementary approaches will be considered:

(i) **Study-specific treatment effect estimation and meta-analysis**: Treatment effects will first be estimated separately within each trial using appropriate models, as detailed in their respective statistical analysis plans. Estimated treatment effects and their standard errors will then be combined using a fixed-effect or random-effects meta-analytic approach, depending on observed heterogeneity.

(ii) **Pooled individual participant data analysis:** A pooled individual participant dataset will be constructed using only Period 1 data from the SMARTEST trial (NCT04405388) and the full dataset from the PolyCAD trial. This approach ensures comparability of treatment groups by restricting the crossover trial to first-period

randomised observations, thereby avoiding potential carryover and period effects. Treatment effects will be estimated using a linear model adjusting for study (trial indicator).

All pooled analyses will be considered exploratory and hypothesis-generating. The primary aim is to assess the consistency of the treatment effect across study designs.

5.12.1 Cardiovascular pooling domain

The primary pooled cardiovascular endpoint will be the change from baseline to end of trial follow-up in 24-hour ambulatory systolic blood pressure (mmHg).
Additional supportive pooled cardiovascular endpoints will include:

- Diastolic blood pressure (mmHg) from 24-hour ambulatory blood pressure
- Heart rate (min^{-1}) from 24-hour ambulatory blood pressure
- Aortic systolic blood pressure (mmHg)
- Systolic dip percentage (%)
- Pulse wave velocity (m/s)
- Augmentation index @75 (%)
- NT-proBNP (ng/L)
- Triglycerides (mmol/L)
- Total cholesterol (mmol/L)
- LDL (mmol/L)
- HDL (mmol/L)
- HbA1c (mmol/mol)

5.12.2 Inflammation pooling domain

The primary pooled inflammation endpoint will be the change from baseline to end of trial follow-up in hsCRP (mg/L).
Additional supportive pooled inflammation endpoints will include:

- IL-6
- TNF-α
- Leukocytes
- Neutrophils
- Eosinophils
- Monocytes
- Lymphocytes
- Neutrophil to lymphocyte ratio
- Markers of autophagy (p62/SQSTM1)

5.12.3 Cardiometabolic pooling domain

To assess the effect of spermidine supplementation compared with placebo on cardiometabolic risk, the change from baseline to end of follow-up in a standardized composite Z-score of five established cardiovascular risk factors (systolic blood pressure, LDL cholesterol, fasting glucose, body mass index (BMI) and hsCRP) will be analysed. The Z-score will be constructed by standardizing each component variable (mean = 0, SD = 1) and summing across the five components, with higher values indicating worse cardiometabolic risk.

6 SAFETY AND TOLERABILITY

6.1 Overall Summary of Tolerability

The safety analysis will be summarized by actual treatment received and analysis will be based on the safety analysis population. Data will be summarized descriptively and stratified by treatment groups.

6.2 Adverse events

Adverse events will be reported from the date of informed consent to the date of study completion (date of follow-up visit). All AEs will be coded to organ class and preferred term.

Treatment emergent adverse events (TEAEs) are defined as adverse events that occur after the date of the first dose of the study drug and before the date of study completion.
The following adverse events will be summarized including counts and percentages stratified by study intervention:

- Any TEAE
- Any study drug related TEAE
- Any serious adverse event (SAE)
- Any treatment emergent serious adverse event (TESAE)
- Any TEAE/TESAE leading to discontinuation of study drug
- Any TEAE/TESAE leading to discontinuation of study participation
- Any TEAE/TESAE leading to death

If the same preferred term is reported more than once for a participant, the participant will be counted once within the corresponding system organ class and preferred-term category using the maximum severity and the closest relationship to the study intervention.

All AEs and TEAEs will be summarized and reported according to treatment group. Events will be graded according to severity (mild, moderate, severe, life-threatening or death) and relationship to the intervention (related or not related). The following summaries will be provided:

- A summary of TEAEs/AEs not related to intervention
- A summary of TESAEs/SAEs not related to intervention
- A summary of TEAEs related to intervention
- A summary of TESAEs related to intervention

Summaries will report patient count and percentage for each event type stratified by treatment group.

6.3 Missing Start and Stop Dates for Adverse Events

Missing start and stop dates for adverse events will be handled according to the algorithm below:

- Missing start dates:
    - If the start day is missing and the month of the adverse event is known, the start day will be set to the first day of the start month of the adverse event.
    - If the start day and month of the adverse event is missing but the year is known, the start date will be set to January 1st.
    - If the start day, month and year of the adverse event is missing, the start date will be set as the day of first dosing.
- Missing end dates:
    - If the end day is missing and the month of the adverse event is known, the end day will be set to the last day of the start month of the adverse event.
    - If the end day and month of the adverse event is missing but the year is known, the end date will be set to December 31st.

- If the end day, month and year of the adverse event is missing, the adverse event will be handled as an ongoing adverse event.

6.4 Summary of Intervention Exposure and Overall Compliance

Compliance will be evaluated at the follow-up visit and total compliance will be summarized. At the follow-up visit participants will be defined as compliant or non-compliant based on the following:

- At the follow-up visit, adherence will be calculated as: (number of capsules dispensed – number of capsules returned)/expected number of capsules administered since randomisation). Participants with ≥80% adherence will be classified as compliant.

6.5 Concomitant and Other Medications

At the follow-up visit concomitant medication will be registered and any changes in medication since baseline will be summarized and reported on the eCRF.

7 REFERENCES

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8 APPENDIX

8.1 Sample R Code

ANCOVA:

```
endpoint_variable_ANCOVA <- dataframe |>
  lm(endpoint_variable_follow-up ~ grp + endpoint_variable_baseline, data = dataframe)
```

Variable	Definition	Role in the ANCOVA model
endpoint_variable	The corresponding endpoint (etc. left ventricular mass)	Dependent variable
grp	This variable defines whether a participant was in the spermidine treatment group or in the placebo group	Fixed effect
endpoint_variable_baseline	Baseline value of the corresponding endpoint variable selected in the model	Covariate

Linear mixed model (sensitivity analysis):

R-packages: lme4, parameters

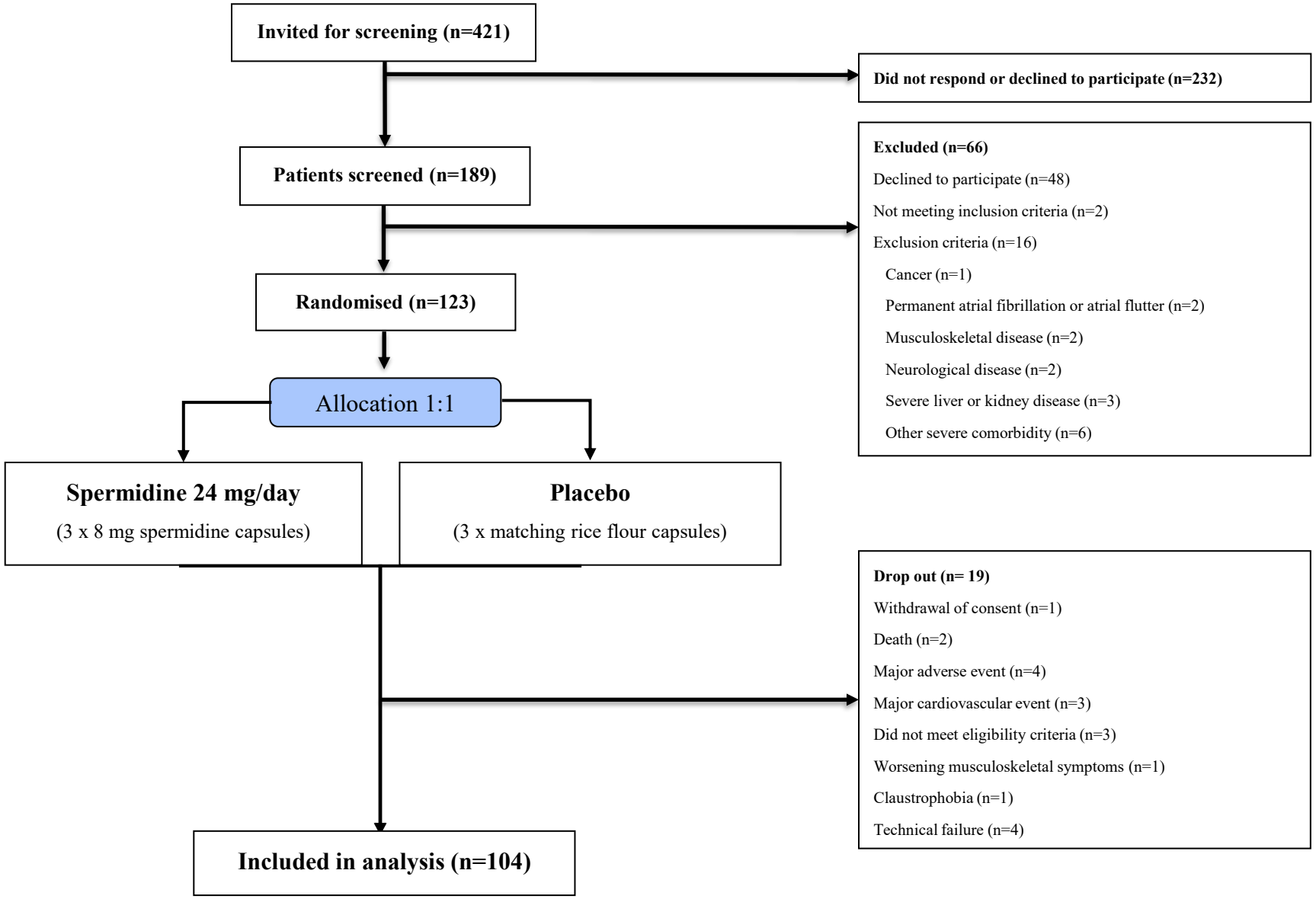
```
endpoint_variable.lmer <- dataframe |>
  lmer(endpoint_variable ~ time*grp+ (1 | study_id), REML = TRUE, na.action = na.exclude,
       control=lmerControl(check.nobs.vs.nRE="ignore"), data = _)

endpoint_variable.lmer |>
  parameters(effects = "fixed", ci_method = "kenward") |>
  print()
```

Variable	Definition	Role in the model
endpoint_variable	The corresponding endpoint (etc. left ventricular mass)	Dependent variable
time	This variable defines the study visit and has two values, baseline or follow-up.	Fixed effect
grp	This variable defines whether a participant was in the spermidine treatment group or in the placebo group	Fixed effect
study_id	Each participants unique study ID	Random effect

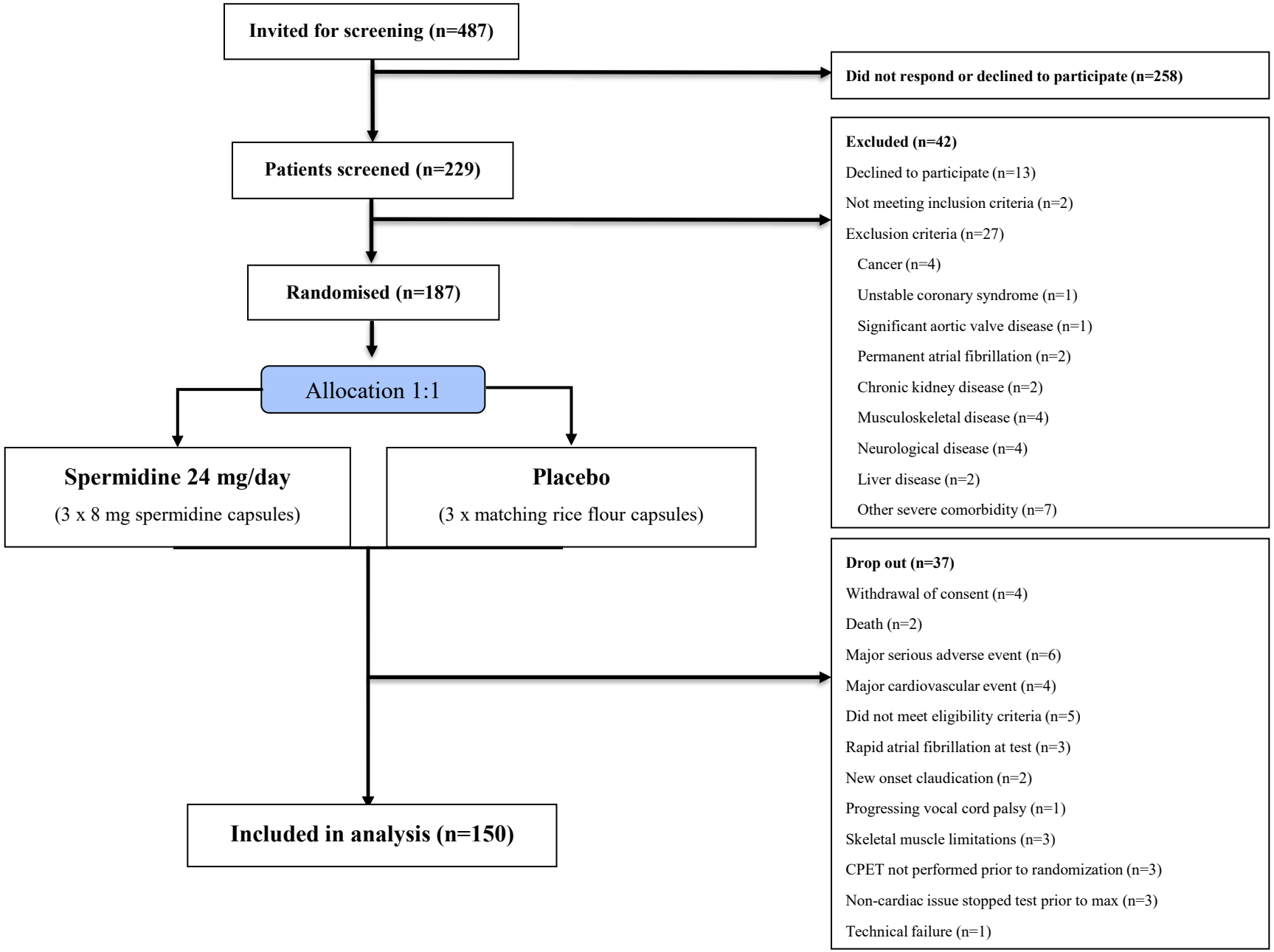
8.2 CONSORT diagrams

CONSORT diagrams for the cardiac remodeling, physical performance, body composition and inflammation domains, as well as for the exploratory cognition domain, are presented below.

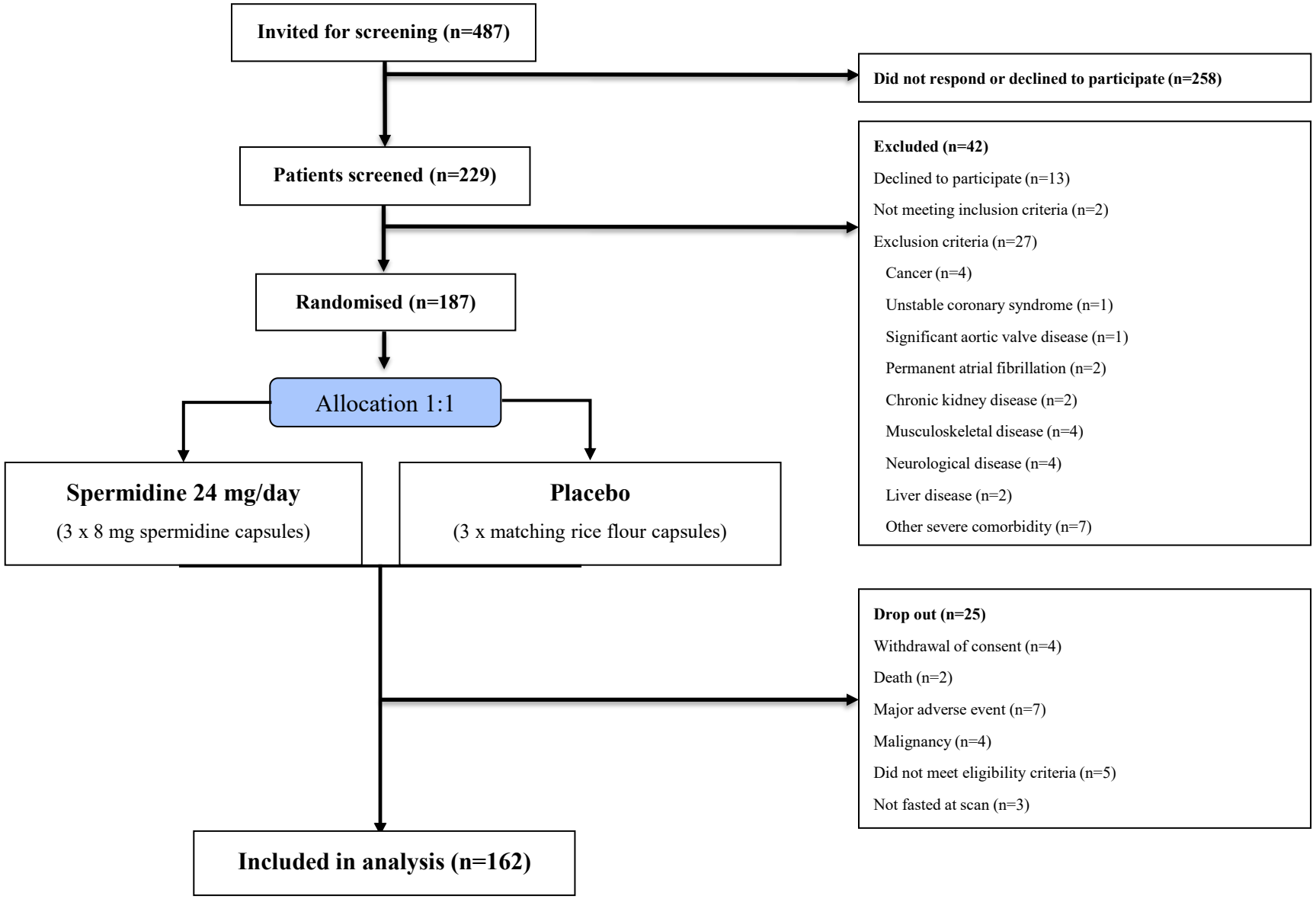
Cardiac remodeling



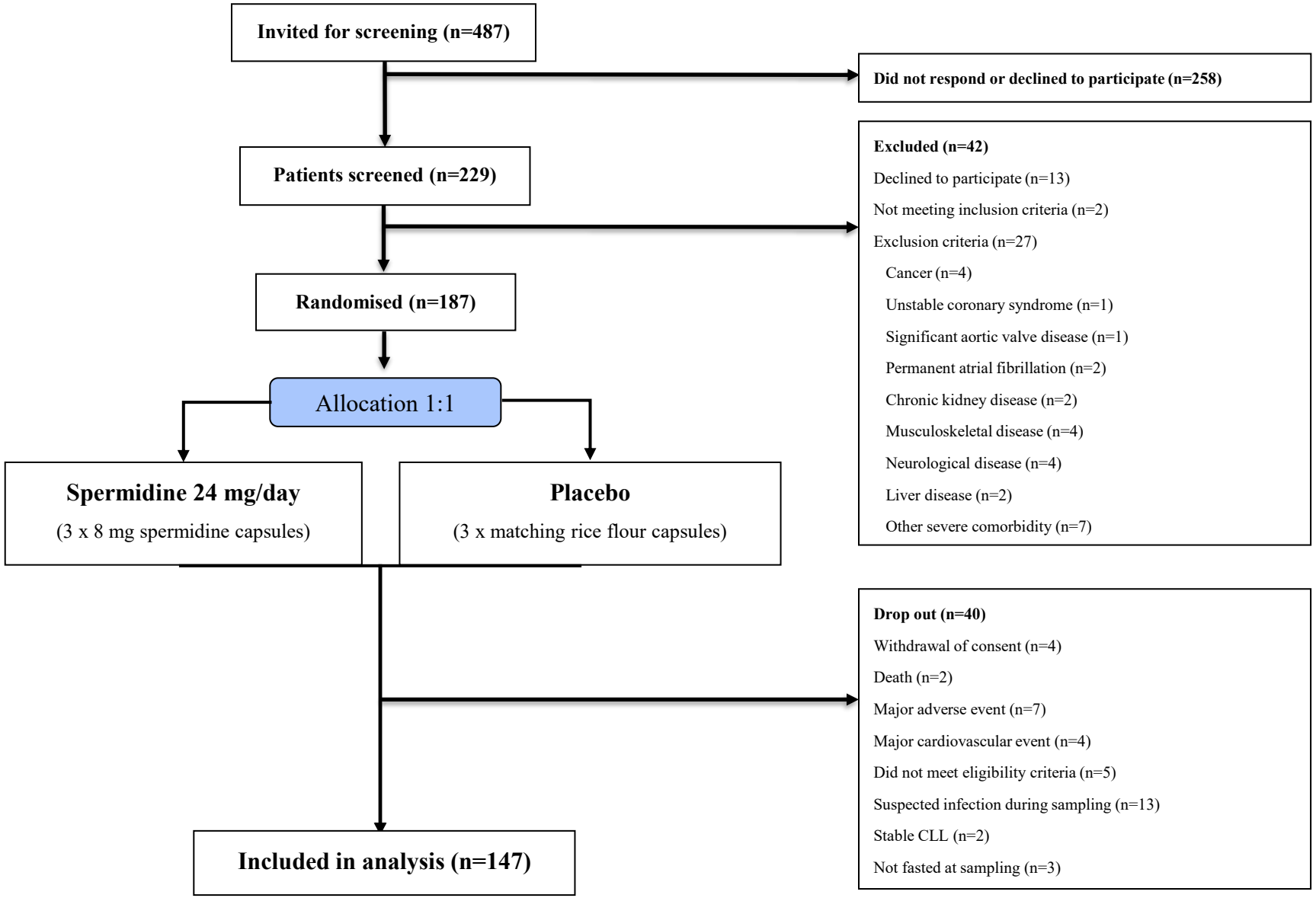
Physical performance



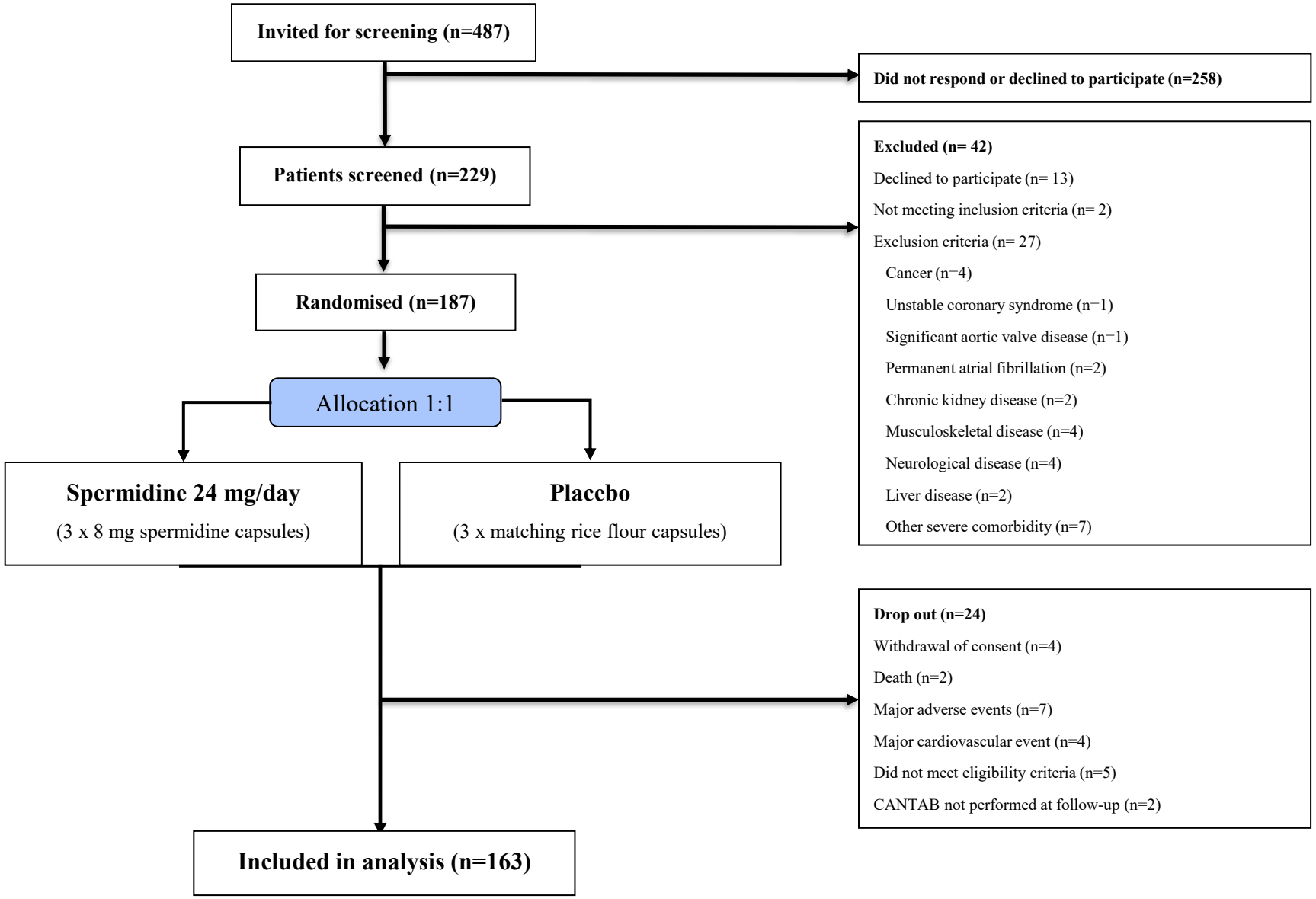
Body composition



Inflammation



Cognition


Invited for screening (n=487)
Did not respond or declined to participate (n=258)
Patients screened (n=229)
Excluded (n= 42)
Declined to participate (n= 13)
Not meeting inclusion criteria (n= 2)
Exclusion criteria (n= 27)
Cancer (n=4)
Unstable coronary syndrome (n=1)
Significant aortic valve disease (n=1)
Permanent atrial fibrillation (n=2)
Chronic kidney disease (n=2)
Musculoskeletal disease (n=4)
Neurological disease (n=4)
Liver disease (n=2)
Other severe comorbidity (n=7)
Randomised (n=187)
Allocation 1:1
Spermidine 24 mg/day
(3 x 8 mg spermidine capsules)
Placebo
(3 x matching rice flour capsules)
Drop out (n=24)
Withdrawal of consent (n=4)
Death (n=2)
Major adverse events (n=7)
Major cardiovascular event (n=4)
Did not meet eligibility criteria (n=5)
CANTAB not performed at follow-up (n=2)
Included in analysis (n=163)