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Complete List of Authors:	DUTHEIL, Frédéric; Occupational Medicine, University Hospital CHU G. Montpied; Australian Catholic University, Faculty of Health, School of Exercise Science CHAPLAIS, Elodie; Australian Catholic University, School of Exercise Science; University of Lorraine; DevAH Laboratory (EA 3450), Exercise Sciences VILMANT, Audrey; Occupational Medicine, University Hospital CHU G. Montpied COURTEIX, Daniel; Laboratoire AME2P - Université Clermont Auvergne; Australian Catholic University, School of Exercise Science DUCHE, Pascale; AME2P Labratory - Université Clermont Auvergne ABERGEL, Armand; Université Clermont Auvergne, CNRS, UMR 6284, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Hepatology Gastroenterology PFABIGAN, Daniela M. ; School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behavior and Mental Health, Peking University HAN, Shihui; School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behavior and Mental Health, Peking University MONDILLON, Laurie; University Clermont Auvergne, and LAPSCO-CNRS, Psychology VALLET, Guillaume T.; Université Clermont Auvergne, CNRS, LaPSCo, Physiological and Psychosocial Stress Mermillod, Martial; Centre Hospitalier Universitaire Grenoble Alpes BOUDET, Gil; Occupational Medicine, University Hospital CHU G. Montpied Obert, Philippe; University of Avignon, Laboratory of Pharm-Ecology Cardiovascular EA4278 Izem, Omar; Laboratory of Cardiovascular Pharm-ecology (LaPEC EA4278), Avignon University MIOLANNE-DEBOUIT, Magalie; University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition Farigon, Nicolas; University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition Pereira, Bruno; University Hospital CHU Clermont-Ferrand, Boirie, Yves; Université Clermont Auvergne, INRA, CRNH, University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition

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ObesiStress: Stress Management in Obesity during a thermal spa residential program – The protocol of a randomized controlled trial study.

Frédéric DUTHEIL[1,2], Elodie CHAPLAIS[2,4,5,15], Audrey Vilmant[1], Daniel COURTEIX[2,3], Pascale DUCHE[3], Armand ABERGEL[6] , Daniela M. PFABIGAN[7], Shihui HAN[7], Laurie MONDILLON[8], Guillaume T. VALLET[8], Martial MERMILLOD[9,10], Gil BOUDET[1], Philippe OBERT[2,11], Omar IZEM[11], Magalie MIOLANNE-DEBOUIT[12], Nicolas FARIGON[12], Bruno PEREIRA[13], Yves BOIRIE[14]

Authors' affiliations:

[1] Université Clermont Auvergne, CNRS, LaPSCo, Physiological and Psychosocial Stress, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Preventive and Occupational Medicine, WittyFit, Clermont-Ferrand, France

[2] Australian Catholic University, Faculty of Health, School of Exercise Science, Melbourne, Victoria 3065 Australia

[3] Université Clermont Auvergne, Laboratory of the Metabolic Adaptations to Exercise under Physiological and Pathological Conditions (AME2P - EA 3533), Clermont-Ferrand, France

[4] Australian Catholic University, Faculty of Health Science, School of Exercise Science, Strathfield, NSW 2135 Australia

[5] University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Biostatistics Unit, Clinical research and Innovation Department (DRCI), Clermont-Ferrand, France

[6] Université Clermont Auvergne, CNRS, UMR 6284, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Hepatology Gastroenterology, Clermont-Ferrand, France

[7] School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behavior and Mental Health, Peking University, Beijing, China

[8] Université Clermont Auvergne, CNRS, LaPSCo, Physiological and Psychosocial Stress, Clermont-Ferrand, France

[9] Grenoble Alpes University, LNPC, CNRS, Grenoble, France

[10] Institut Universitaire de France, Paris, France

[11] Laboratory of Cardiovascular Pharm-ecology (LaPEC EA4278), Avignon University, Avignon, France

[12] University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition, Clermont-Ferrand, France.

[13] University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Biostatistics Unit, Clinical research and Innovation Department (DRCI), Clermont-Ferrand, France

[14] Université Clermont Auvergne, INRA, CRNH, University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition, Clermont-Ferrand, France.

[15] Université de Lorraine, Laboratory ``Development, Adaption and Disability'' (DevAH - EA 3450), Nancy, France

- Corresponding author – CHAPLAIS Elodie (PhD), Université de Lorraine, Laboratory ``Development, Adaption and Disability'' (EA 3450), Faculty of Sports Sciences and Faculty of Medicine, 9 avenue de la Forêt de Haye, CS 50184, F-54505 Vandœuvre-lès-Nancy, France; elodie.chaplais@acu.edu.au

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Abstract - Introduction: Stress and obesity are two public health issues. The relationships between obesity and stress are biological via the action of stress on the major hormones regulating appetite (leptin, ghrelin). Many spa resorts are specialized in the treatment of obesity in France but actually no thermal spa proposes a specific program to manage stress in obesity. The ObesiStress protocol was designed to offer thermal spa residential program of stress management in the treatment of obesity, which implement stress management strategies as suggested by International recommendations.

Methods and analysis: 140 overweight or obese participants with Body Mass Index (BMI) >25 kg.m-2 and aged over 18 years will be recruited. Participants will be randomized into two groups: a control group of usual practice (restrictive diet, physical activity and thermal spa treatment) and an intervention group with a stress management in addition to the usual practice. In the present protocol, parameters will be measured on five occasions (at inclusion, at the beginning of the spa (day 0), at the end of the spa (day 21), at 6 and 12 months). The study will assess participants' heart rate variability, cardiac remodeling and function, electrodermal activity, blood markers, anthropometry profile, body composition, psychology and quality of life via questionnaires and bone parameters.

Ethics and dissemination: The ObesiStress protocol complies with the Ethics guidelines for Clinical Research and has been approved by the Ethic Committee (CPP Sud-Est VI, Clermont-Ferrand - ANSM: 2016-A01774-47). This study would highlight the effect of a 21 days thermal spa residential program of stress management in obesity will exhibit its efficacy through objective measures of well-being and cardiovascular morbidity. Results will be disseminated at several research conferences and published articles in peer-reviewed journals.

Trial Registration: NCT 03578757

Strengths and limitations of this study:

- Reliability and validity of a 21 days specific thermal spa residential program on stress management in the treatment of obesity
- Better comprehension of psychological and psychological mechanisms involved in stress management in obesity.
- 12-months mixt gender longitudinal study
- Unable to accurately account for the long-term cost-effective benefits of the study

Introduction

Stress and obesity are two public health issues.[1-3] Stress can lead to obesity via inappropriate eating behaviors.[4] In addition, obesity is a major stress factor.[5] Furthermore, stressed people are also those who have the greatest difficulties to lose weight.[6] The relationships between obesity and stress are biological via the action of stress on the major hormones regulating appetite (leptin, ghrelin).[7, 8] The relationships between obesity and stress are so strong that proposals of international recommendation suggest to implement stress management programs in obesity for a sustainable weight loss.[9]

Among the multiple physical and psychological consequences of stress and obesity, increased mortality and cardiovascular morbidity seem the main concern.[10] Stress and obesity alter the functioning of the autonomic nervous system. [11-13] A deregulation of sympatho-vagal balance is a major factor of morbidity cardiovascular mortality.[14] Stress and obesity also cause arterial ischemic pathology, [15] via complex mechanisms involving changes of endothelial and arterial atherosclerosis.[15] These microvascular changes are linked to systemic inflammation caused by stress[16] and obesity.[17]

The thermal spa resort of Vichy, as well as other some spa centers in France, have already an expertise in obesity treatment by physical activity, diet, and hydrotherapy. However, there are no spa resort centers which has ever proposed to include a stress management program in obesity treatment. Non-pharmacological stress management can be done through psychological interventions (i.e. physical [18] and psychoanalytic approaches [19], cognitive behavioral therapy,[20] acceptance and commitment therapy,[21, 22] or mindfulness.[23, 24]), physical activity,[25, 26] and improvement of eating disorders induced by stress.[27] The benefits of physical activity on the physical and mental health are indisputable, at any age, and with any activity.[28]

The main hypothesis of this project is that a thermal spa residential program (21 days) of stress in obesity will exhibit its efficacy through objective measures of well-being and cardiovascular morbidity, such as heart rate variability which is both a biomarker of stress and linked with life expectancy.

Methods

Protocol design: This one-year randomized controlled study with repeated measures on five occasions (inclusion, at the start, at the end of the spa, at 6 and 12 months), will allow us to understand the effect of a 21 days spa residential program of stress management in the treatment of obesity especially through measures of well-being and cardiovascular morbidity.

For this study, two groups of overweight or obese participants will be compared: one receiving a 21 days usual thermal spa residential program, one receiving a 21 days usual thermal spa residential program plus a psychological intervention (Figure1). Randomization will be stratified by BMI category (25-30, 30-35, >35), sex, and level of stress (visual analog scale of stress <50, between 50 and 80, >80), using minimization approach.

Selection criteria:

Inclusion criteria common to studies: Volunteers will be overweight or obese participants aged over 18 years, who wish to follow a spa thermal residential program for the treatment of obesity. Participants must have a stable weight during the last three months, with no hepatic, renal or endocrine diseases uncontrolled. Stress at baseline will not be an inclusion criteria but an explanatory/independent variable. In compliance with Human Ethics guidelines, participants will have to be covered by a social health insurance and will have to sign consent forms.

Exclusion criteria: Volunteers participating in the study will be excluded if major treatment and/or protocol deviations are observed.

Power analysis: The rationale for the sample size calculation is based on (HRV) the log LF / HF with low values associated with a good adaptation of the autonomic nervous system. We considered a comparison of two randomized groups with the same sample size, with a statistical power at 90%, and a two-sided type I error of 5%. According to our results from a pilot study, [3] we assumed that log LF / HF would decrease the by 25±22% in the group with a stress management program vs 13±20% in the group without a stress management program. Assuming a difference of 12±20% between the two groups at one year, we need to include n = 59 participants by group. Finally, to take into account lost to follow-up, it is proposed to recruit 70 patients per arm.

Participants: As previously stated participants engaged in this protocol will be mixed gender overweight or obese volunteers aged over 18 years. Following approval from Ethic committees, and based on our calculation, a total of 70 volunteers per group (i.e. 140 in total) will be enrolled to account for potential dropouts. All participants will be given written information regarding the project and will have to signed consent forms before enrollment. Patients will be recruited through the usual participants of the spa resort of Vichy, through health-care workers (physicians, dieticians, physiotherapist, etc.), or through advertisements. Inclusions will be realized at the CHU Clermont-Ferrand or at the thermal spa resort of Vichy.

Usual thermal spa care: All participants will benefits a usual practice thermal spa treatment combining corrections of eating disorders (and a negative energy balance of 500 kcal/day), physical activity (2h30 per day, minimum), thermal spa treatment (2h per day, minimum), and health education (1h30 per day, minimum: cooking, nutrition and physical activity classes…). Physical activity will be diverse (endurance, strength, circuit training) and personalized to the target of each participant.

Psychological interventions: Participants randomized to the intervention group will benefit from psychological interventions based on validated approaches of stress (3 x 1h30 per week). Participants will attend psychological sessions by group of less than 10 individuals. Individual meeting with the psychologist will occur at least twice: at the beginning of the residential program and at the end. Psychological interventions will include various validated approaches of work-related stress: physical [18] and psychoanalytic [19] approaches, cognitive behavioral therapy,[20] acceptance and commitment therapy,[22, 29] mindfulness,[23, 24] etc. Participants will have to acquire techniques in order to be autonomous and pursue at-home psychological training.

Follow-up: After the intervention phase of the study, participants will undergo a one-year at-home follow-up.

Measurements: Each participants will perform a battery of tests (described below). Data collection will be performed 5 times as previously described, except for DXA and pQCT which will be performed at inclusion and after 12 months, cardiac remodeling and function which will be performed at inclusion and after 6 months.

Primary outcome: To assess the ability of a 21 days spa residential program of stress management in the treatment of obesity on increasing heart rate variability, a biomarker of both stress and morbidity/mortality.

Heart rate variability: HRV parameters will be assessed during 26 hours with a heart rate transmitter belt simply positioned on the chest, with a 26h recording time, a beat per minute within a 25-240 range, and respiratory rate within a 3-70 range (Zephyr™ BioHarness™ BT, Zephyr Technology, Annapolis, USA). The HRV data will be examined according to the recommendations of the European Society of Cardiology and the North American Society (Task Force). HRV will be explored in time and frequency domains. [30] The methodology developed by our team will also be applied.[31]

Secondary outcomes: Secondary outcomes will be (1) to demonstrate an improvement in stress- and obesity-related variables following the short spa residential program, (2) to study the influence of genetic polymorphisms on stress, obesity, and on the response to our stress management program, (3) to examine the relationship between stress- and obesity-related variables, (4) to propose a salient biomarker or a salient composite index of biomarkers of stress in obesity and (5) to study the effect of observance to the program during the follow-up on stress- and obesity-related variables.

Anthropometry: Anthropometric measures will be taken according to the recommendations of the International Society for the Advancement of Kinanthropometry for the following measures: standing height *(m)* and body mass *(kg)*, waist circumference *(cm)*, and lower limb bone lengths/breadths *(cm)*[32].

Body Composition: Body composition (muscle mass and fat mass) will be measured by DXA (DXA, QDR-4500A, Hologic, Inc., Waltham, MA).

Biomarkers of stress and cardiovascular risk: Skin conductance will be measured using wrist band electrodes with sampling rates at 2, 4, 8, 16 and 32 Hz during the phases 1 to 3. The SC sensor (Q-Sensor®-

Affectiva®, Massachusetts Institute of Technology, USA) is set on a wristband and has a 24-hour battery life when logging. In addition, it will measure wrist movements with a build in 3-Axis accelerometer.

Blood flow velocity and myocardial longitudinal strain will be measured by Speckle echocardiography (Vivid Q, GE Healthcare, USA). All 2D-dimensionnal, time-motion, Doppler and 2D-strain acquisitions and measurements will be performed according to recent guidelines. [33, 34] Left ventricular (LV) volumes and ejection fraction (EF) will be measured using the Simpson's biplane method. LV mass (LVM) will be calculated by the Devereux formula and indexed for height (Cornell adjustment). Pulsed-Doppler LV transmitral velocities, including early (E) and atrial (A) waves, will be obtained in the apical 4-chamber view. Tissue Doppler Imaging (TDI) measures of myocardial systolic (S'), early diastolic (E') and atrial (A') velocities will be assessed at the mitral annulus level in the apical 4- and 2-chamber views. The E/Em ratio was used as an index of LV filling pressure 85. Left atrium (LA) volume was assessed on apical 4- and 2 chamber views. A graduation of LV diastolic dysfunction will be obtained according to recent guidelines 85. 2D cine-loops (frame rate >70ips) of at least 5 cycles will recorded in the short-axis views (base, md, apex), as well as in the apical 4-, 3- and 2-chambers views. 2D-strain analysis will be performed post-processing using EchoPAC 201TM software (GE Healthcare, USA). Longitudinal and circumferential strains and strain rates (SR), as well as rotations at the apex and base, will be directly obtained from the 6 segment model. Twist mechanics will be computed from apical and basal rotational data using dedicated software (Scilab, Paris, France). For each view, the 3 cardiac cycles displaying the best image quality will be selected. Blood pressure and heart rate will be continuously monitored, and the systolic meridional wall stress, an index of afterload, will be calculated. LV end-diastolic volumes will also be obtained as preload index.

Endocrine assays: Blood samples will be collected by a qualified pediatric nurse after participants have fasted overnight. Blood will be collected using a venipuncture at the brachial vein. After collection, blood will be centrifuged and aliquots will be stored *(-80°)* for subsequent analysis.

Basic biology *(TG, cholesterol, LDLC, HDLC, HbA1c, cortisol, DHEAS)* as well as all other biochemical determination *(leptin, ghrelin, BDNF, IL-1β, IL-6, IL-1, TNFα, NPY)* will be assessed in the biochemistry laboratory of Clermont-Ferrand University Hospital. All analyses will be conducted by the same technician. Polymorphism of the angiotensin converting enzyme and polymorphism of the serotonin will be measured by blood cells.

Complementary measures: Stress fatigue and sleep (Visual analogue scale of 100 mm), burn out (Maslach Burn Out Inventory), depression and anxiety (Hospital Anxiety and Depression Scale, STAI-Y and Hamilton scale for anxiety 7 items), mindfulness (Freiburg Mindfulness Inventory), coping strategies (Brief COPE Questionnaire), emotions (Emotion Regulation Questionnaire), perception of work (Job Content Questionnaire of Karasek), self-efficacy (Perceived self-efficacy scale), alexithymia (Toronto Alexithymia Scale), illness perception (Brief Illness Perception Questionnaire (B-IPQ)), metacognition (MetaCognition Questionnaire), general health (General health questionnaire 12 items), lifestyle (smoking, alcohol…), and physical activity (Recent Physical Activity Questionnaire (RPAQ)) will be obtained by questionnaires.

Bone parameters: Bone microarchitecture will be measured by Peripheral Quantitative Computed Tomography (pQCT, XCT 3000 Stratec Medizintechnik Pforzheim, Germany).[35-37] Bone mineral content *(g/cm)*, volumetric cortical and trabecular BMC *(mg/cm^3)*, total area *(mm^2)*, cortical and trabecular area *(mm^2)* and density *(g/cm^2)*, bone strength *(mm^3)*, will be assessed by tomographic slices of 2 mm thickness at the distal (4%), proximal (66%) site of the non-dominant tibia and radius. Scan speed and voxel size are 20 mm/s and 0.4 mm, respectively. To assure quality of measurement, calibration checks will be performed by scanning a standard phantom with known densities, prior to each scan.

Bone densitometry will be measured by Dual energy X ray Absorptiometry (DXA, QDR-4500A, Hologic, Inc., Waltham, MA). Bone mineral density (BMD, g/cm2), bone mineral content (BMC, g), bone area (cm2) will be determined for each participant. The DXA measurements will be taken for whole body, lumbar spine (L2-L4) and non-dominant hip (including the femoral neck, trochanteric and intertrochanteric regions). All DXA scans will be conducted by the same technician and quality assurance checks will be performed routinely. The in-vivo coefficient of variation (CV) is 0.5%.

Statistical analysis: Statistical analysis will be performed using Stata software (version 13; Stata-Corp, College Station, Tex., USA). All statistical tests will be two-sided and $p<0.05$ will be considered significant. After testing for normal distribution (Shapiro-Wilk test), data will be treated either by parametric or non-parametric analyses according to statistical assumptions. Inter-groups comparisons will be performed 1) without adjustment and 2) adjusting on possible confounder's factors.

To highlight that the spa residential program will have long-term benefits (one year) on biomarkers of stress, the comparisons will be performed using Student t-test or Mann-Whitney test if assumptions of t-test are not respected (normality and homoscedasticity analyzed using Fisher-Snedecor's test). The results will be expressed as effect-size and 95% confidence interval. This primary analysis will be completed by multivariable analysis (linear regression with logarithmic transformation of dependent outcome if necessary) considering an adjustment on covariates fixed according to univariate results, epidemiological relevance and observance to physical activity. The results will be expressed as regression coefficients and 95% confidence intervals.

Comparisons between groups will be performed similarly as presented previously for quantitative outcomes. Comparisons concerning categorical variables will be performed using Chi-squared or when appropriate Fischer-exact test. The results will be expressed as absolute risk differences and 95% confidence intervals. Then, the multivariable analysis will be conducted using linear and generalized linear

models according to the statistical nature of dependent endpoint. The results will be expressed as regression coefficients or relative risks, and 95% confidence intervals.

Moreover, the relations between quantitative parameters will be analyzed using correlation coefficients (Pearson or Spearman according to statistical distribution). Considering the several multiple comparisons, a correction of the type I error will be applied (Sidak's correction). The comparisons of correlation coefficients (in different groups of subjects and within a single group of subjects) will be performed using Fisher's Z transformation [38] and Williams' T2 statistic [39]. Multidimensional factorial analyses will be performed to complete these statistical analyses.

Concerning the parameters collected longitudinally, mixed models will be performed to study fixed effects – group, time points evaluation and their interaction – taking into account between and within subject variability (as random-effect). For continuous endpoints, the normality of residuals will be assessed using the Shapiro-Wilk test.

Radiation

Both DXA and pQCT provide measures of body composition and bone properties by exposing participants to low level radiation: 0.0056 mSv from DXA scans *(whole body, lumbar and hip)* and 0.0014 mSv from the pQCT scans *(tibia and radius measures)* [40]. Over the duration of each study, the effective dose of 0.014 mSv will be administered.

Patient and public involvement

The Thermal spa center of Vichy in collaboration with the Preventive and Occupational Medicine Department of the University Hospital of Clermont-Ferrand identified and addressed the following priorities: prevention, obesity, weight loss, stress, cardiovascular morbidity. We are grateful for the opinion of volunteers and professionals from the Vichy Spa Center on the psychological intervention. Conferences and meetings with participants will be organised in order to provide them feedback from this research.

Ethical considerations and dissemination

The ObesiStress protocol complies with the Ethics guidelines for Clinical Research and has been approved by the Ethic Committee (CPP Sud-Est VI, Clermont-Ferrand - ANSM: 2016-A01774-47), the protocol has also been registered in Clinical Trial NCT 03578757. In accordance with Ethical considerations, the chief investigator is responsible of ensuring that participants understand potential risks and benefits of taking part in the study. Moreover, the chief investigator is responsible for obtaining writing consent from participants. The results will be disseminated at several research conferences and published articles in peer-reviewed journals.

Discussion

The ObesiStress protocol was designed to provide a better understanding of the effect of a spa residential program combined with a stress management program on the improvement of heart rate variability in the treatment of obesity. The creation of a new thermal program would allow new innovative approaches for stress management in obesity. The long-term success of lifestyle interventions such as those proposed in prevention of obesity is the observance to the treatment (nutrition, physical activity, psychology).[41] We previously demonstrated that a spa program may play a major role in sustainable lifestyle changes.[42] Because of the stress management program and because the participants will be accompanied by health-care professionals, the observance to treatment during a one-year follow-up could be more efficient.

Current study status

The status of the trial is ongoing recruitment.

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Competing interests

The authors declare that they have no competing interests.

Author's contributions

FD contributed to the study design. FD was responsible for ethics committee. FD and AV will coordinate the recruitment of patients. DC, EC and PD will be responsible for bone measures. AA will be responsible for liver-related factors. DP and SH will be responsible for psychosocial analysis. LM and MM will be responsible for mindfulness, psychological education and cognitive-compartmental therapy analysis. GV will be responsible for the analysis of memory-related data. PO, OI and GB will be responsible for cardiovascular measures. YB, NF and MMD will be responsible for nutritional measures and analysis. DC, EC and PD will be responsible for physical activity analysis. AV will be responsible for the collection of psychological factors. BP is responsible for the statistical analysis. EC was responsible for the clinical trial and wrote the first draft of this manuscript. All authors read, contributed towards and approved of the final manuscript.

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Nothing to declare

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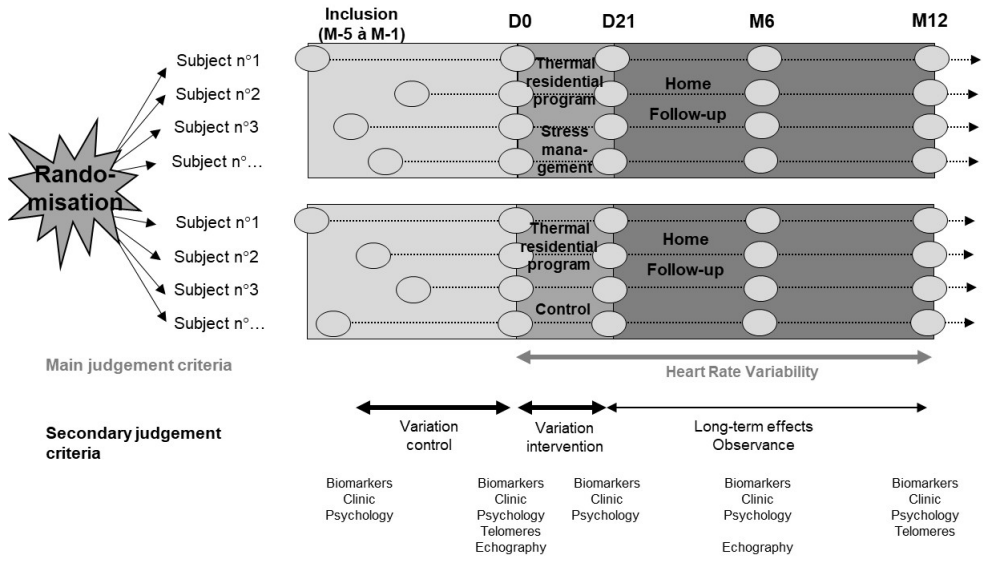
Figure Title: ObestiStress protocol

Figure Abbreviation: M months, D days



Title: The ObesiStress protocol
M months, D day

105x59mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	Page 2
Funding	#4	Sources and types of financial, material, and other support	Page 14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Pages 1-2;14
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	Page 2

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Page 14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	
Objectives	#7	Specific objectives or hypotheses	Page 5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	Page 6
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Pages 7-8

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Pages 8-11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Pages 6, 8, Figure 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Pages 6-7
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 7
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 6
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Pages 8-11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Pages 11-12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Pages 11-12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple	Pages 11-12

		imputation)	
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	Page 24
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	
Declaration of	#28	Financial and other competing interests for principal	Page 14

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 13
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

BMJ Open

ObesiStress: Stress Management in Obesity during a thermal spa residential program – The protocol of a randomized controlled trial study.

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Complete List of Authors:	DUTHEIL, Frédéric; Occupational Medicine, University Hospital CHU G. Montpied; Australian Catholic University, Faculty of Health, School of Exercise Science CHAPLAIS, Elodie; Australian Catholic University, School of Exercise Science; University of Lorraine; DevAH Laboratory (EA 3450), Exercise Sciences VILMANT, Audrey; Occupational Medicine, University Hospital CHU G. Montpied COURTEIX, Daniel; Laboratoire AME2P - Université Clermont Auvergne; Australian Catholic University, School of Exercise Science DUCHE, Pascale; AME2P Labratory - Université Clermont Auvergne ABERGEL, Armand; Université Clermont Auvergne, CNRS, UMR 6284, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Hepatology Gastroenterology PFABIGAN, Daniela M. ; School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behavior and Mental Health, Peking University HAN, Shihui; School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behavior and Mental Health, Peking University MONDILLON, Laurie; University Clermont Auvergne, and LAPSCO-CNRS, Psychology VALLET, Guillaume T.; Université Clermont Auvergne, CNRS, LaPSCo, Physiological and Psychosocial Stress Mermillod, Martial; Centre Hospitalier Universitaire Grenoble Alpes BOUDET, Gil; Occupational Medicine, University Hospital CHU G. Montpied Obert, Philippe; University of Avignon, Laboratory of Pharm-Ecology Cardiovascular EA4278 Izem, Omar; Laboratory of Cardiovascular Pharm-ecology (LaPEC EA4278), Avignon University MIOLANNE-DEBOUIT, Magalie; University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition Farigon, Nicolas; University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition Pereira, Bruno; University Hospital CHU Clermont-Ferrand, Boirie, Yves; Université Clermont Auvergne, INRA, CRNH, University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition

Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Nutrition and metabolism, Sports and exercise medicine, Public health
Keywords:	obesity, stress, prevention, heart rate variability, spa bath

ObesiStress: Stress Management in Obesity during a thermal spa residential program – The protocol of a randomized controlled trial study

Frédéric DUTHEIL[1,2], Elodie CHAPLAIS[2,4,5,15], Audrey Vilmant[1], Daniel COURTEIX[2,3], Pascale DUCHE[3], Armand ABERGEL[6] , Daniela M. PFABIGAN[7], Shihui HAN[7], Laurie MONDILLON[8], Guillaume T. VALLET[8], Martial MERMILLOD[9,10], Gil BOUDET[1], Philippe OBERT[2,11], Omar IZEM[11], Magalie MIOLANNE-DEBOUIT[12], Nicolas FARIGON[12], Bruno PEREIRA[13], Yves BOIRIE[14]

Authors' affiliations:

[1] Université Clermont Auvergne, CNRS, LaPSCo, Physiological and Psychosocial Stress, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Preventive and Occupational Medicine, WittyFit, Clermont-Ferrand, France

[2] Australian Catholic University, Faculty of Health, School of Exercise Science, Melbourne, Victoria 3065 Australia

[3] Université Clermont Auvergne, Laboratory of the Metabolic Adaptations to Exercise under Physiological and Pathological Conditions (AME2P - EA 3533), Clermont-Ferrand, France

[4] Australian Catholic University, Faculty of Health Science, School of Exercise Science, Strathfield, NSW 2135 Australia

[5] University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Biostatistics Unit, Clinical research and Innovation Department (DRCI), Clermont-Ferrand, France

[6] Université Clermont Auvergne, CNRS, UMR 6284, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Hepatology Gastroenterology, Clermont-Ferrand, France

[7] School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behavior and Mental Health, Peking University, Beijing, China

[8] Université Clermont Auvergne, CNRS, LaPSCo, Physiological and Psychosocial Stress, Clermont-Ferrand, France

[9] Grenoble Alpes University, LNPC, CNRS, Grenoble, France

[10] Institut Universitaire de France, Paris, France

[11] Laboratory of Cardiovascular Pharm-ecology (LaPEC EA4278), Avignon University, Avignon, France

[12] University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition, Clermont-Ferrand, France.

[13] University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Biostatistics Unit, Clinical research and Innovation Department (DRCI), Clermont-Ferrand, France
[14] Université Clermont Auvergne, INRA, CRNH, University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition, Clermont-Ferrand, France.
[15] Université de Lorraine, Laboratory ``Development, Adaption and Disability'' (DevAH - EA 3450), Nancy, France

Corresponding author – CHAPLAIS Elodie (PhD), Université de Lorraine, Laboratory ``Development, Adaption and Disability'' (EA 3450), Faculty of Sports Sciences and Faculty of Medicine, 9 avenue de la Forêt de Haye, CS 50184, F-54505 Vandœuvre-lès-Nancy, France; elodie.chaplais@acu.edu.au

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Number of figure: 1

Protocol version: version 1, 10/2018

Abstract

Introduction: Stress and obesity are two public health issues. The relationships between obesity and stress are biological via the action of stress on the major hormones regulating appetite (leptin, ghrelin). Many spa resorts are specialized in the treatment of obesity in France but actually no thermal spa proposes a specific program to manage stress in obesity. The ObesiStress protocol was designed to offer thermal spa residential program of stress management in the treatment of obesity, which implement stress management strategies as suggested by International recommendations.

Methods and analysis: 140 overweight or obese participants with Body Mass Index (BMI) >25 kg.m-2 and aged over 18 years will be recruited. Participants will be randomized into two groups: a control group of usual practice (restrictive diet, physical activity and thermal spa treatment) and an intervention group with a stress management in addition to the usual practice. In the present protocol, parameters will be measured on five occasions (at inclusion, at the beginning of the spa (day 0), at the end of the spa (day 21), at 6 and 12 months). The study will assess participants' heart rate variability, cardiac remodeling and function, electrodermal activity, blood markers, anthropometry profile, body composition, psychology and quality of life via questionnaires and bone parameters.

Ethics and dissemination: The ObesiStress protocol complies with the Ethics guidelines for Clinical Research and has been approved by the Ethic Committee (CPP Sud-Est VI, Clermont-Ferrand - ANSM: 2016-A01774-47). This study would highlight the effect of a 21 days thermal spa residential program of stress management in obesity will exhibit its efficacy through objective measures of well-being and cardiovascular morbidity. Results will be disseminated at several research conferences and published articles in peer-reviewed journals.

Trial Registration: NCT 03578757

Strengths and limitations of this study:

- Reliability and validity of a 21 days specific thermal spa residential program on stress management in the treatment of obesity
- Better comprehension of psychological and psychological mechanisms involved in stress management in obesity.
- 12-months mixt gender longitudinal study
- Unable to accurately account for the long-term cost-effective benefits of the study

Introduction

Stress and obesity are two public health issues.[1 2] Stress can lead to obesity via inappropriate eating behaviors.[3] In addition, obesity is a major stress factor.[4] Furthermore, stressed people are also those who have the greatest difficulties to lose weight.[5] The relationships between obesity and stress are biological via the action of stress on the major hormones regulating appetite (leptin, ghrelin).[6 7] The relationships between obesity and stress are so strong that proposals of international recommendation suggest to implement stress management programs in obesity for a sustainable weight loss.[8]

Among the multiple physical and psychological consequences of stress and obesity, increased mortality and cardiovascular morbidity seem the main concern.[9] Stress and obesity alter the functioning of the autonomic nervous system. [10-12] A deregulation of sympatho-vagal balance is a major factor of morbidity cardiovascular mortality.[13] Conceniently, the sympatho-vagal balance can be measured easily and pain-free by heart rate variability (HRV), which is a biomarker of both stress and morbidity/mortality.[10 14 15] Stress and obesity also cause arterial ischemic pathology, [16] via complex mechanisms involving changes of endothelial and arterial atherosclerosis.[16] These microvascular changes are linked to systemic inflammation caused by stress[17] and obesity.[18]

The thermal spa resort of Vichy, as well as other some spa centers in France, have already an expertise in obesity treatment by physical activity, diet, and hydrotherapy. However, there are no spa resort centers which has ever proposed to include a stress management program in obesity treatment. Non-pharmacological stress management can be done through psychological interventions (i.e. physical [19] and psychoanalytic approaches [20], cognitive behavioral therapy,[21] acceptance and commitment therapy,[22 23] or mindfulness.[24 25]), physical activity,[26 27] and improvement of eating disorders induced by stress.[28] The benefits of physical activity on the physical and mental health are indisputable, at any age, and with any activity.[29]

The main hypothesis of this project is that a thermal spa residential program (21 days) of stress management in obesity will exhibit its efficacy through objective measures of well-being and cardiovascular morbidity, via a randomised controlled design comparing a group with stress management and a group without stress management (both groups will benefit from the same spa treatments, physical activity, and diet).

Objectives

The main objective will be to assess the ability of a 21 days spa residential program of stress management in the treatment of obesity on increasing heart rate variability, a biomarker of both stress and morbidity/mortality.

Secondary outcomes will be (1) to demonstrate an improvement in stress- and obesity-related variables following the short spa residential program, (2) to study the influence of genetic polymorphisms on stress, obesity, and on the response to our stress management program, (3) to examine the relationship between stress- and obesity-related variables, (4) to propose a salient biomarker or a salient composite index of biomarkers of stress in obesity and (5) to study the effect of observance to the program during the follow-up on stress- and obesity-related variables.

Methods

Protocol design: This one-year randomized controlled study with repeated measures on five occasions (inclusion, at the start, at the end of the spa program, at 6 and 12 months), will allow us to understand the effect of a 21 days spa residential program of stress management in the treatment of obesity especially through measures of well-being and cardiovascular morbidity.

For this study, two randomized groups of overweight or obese participants will be compared: one receiving a 21 days usual thermal spa residential program, and one receiving a 21 days usual thermal spa residential program plus a psychological intervention (Figure1).

Randomisation

Randomization will be stratified by BMI category (25-30, 30-35, >35), sex, and level of stress (visual analog scale of stress <50, between 50 and 80, >80), using minimization approach. A permuted-block randomization (i.e. random block sizes) will be conducted using a computer-generated random allocation (Stata software, version 13, StataCorp, College Station, US), with a 1:1 ratio allocation,

ensuring complete randomness of the assignment of a participant to each randomized group. To guarantee concealment of allocation, the participants will be randomized after it is clear that they have met the inclusion criteria and have provided written consent.

Selection criteria:

Inclusion criteria: Volunteers will be overweight or obese participants aged over 18 years, who wish to follow a spa thermal residential program for the treatment of obesity. We will also promote the study by advertisements in local newspaper and on radio. Volunteers will be screened by phone interview or directly by spa physicians. Participants must have a stable weight during the last three months, with no cardiac, hepatic, renal or endocrine diseases uncontrolled.[2] Stress at baseline will not be an inclusion criteria but an explanatory/independent variable. In compliance with Human Ethics guidelines, participants will have to be covered by a social health insurance and will have to sign consent forms.

Exclusion criteria: Volunteers participating in the study will be excluded if major treatment and/or protocol deviations are observed.[14] Drugs and medical conditions that significantly affect the primary outcome (heart rate variability) will also be exclusion criteria (e.g. alpha or beta-blockers; arrhythmias or conduction disorders such as bundle branch block, atrioventricular heart block).[30] Bariatric surgery is also an exclusion criteria.

Power analysis: The rationale for the sample size calculation is based on HRV which is a biomarker of both stress and morbidity/mortality.[10 14 15] In particular, within multiple parameters of HRV, we considered the log Low-Frequency/High-Frequency (LF/HF) for sample size calculation because it is the parameter that traditionally represents sympathovagal balance (see description of LF/HF below in the description of the primary outcome section).[10 14] The log LF/HF with low values associated with a good adaptation of the autonomic nervous system. According to our results from a pilot study (data not published),[2] we hope to highlight an absolute difference of 12% between groups concerning the decrease of log LF/HF at one year after the stress management program. For a standard-deviation at 20%, the expected size will be around 0.60. For a two-sided type I error of 5%, we need to include 59 participants

by group to achieve a statistical power equals 90%. Finally, to take into account lost to follow-up, it is proposed to recruit 70 patients per arm.

Participants: As previously stated participants engaged in this protocol will be mixed gender overweight or obese volunteers aged over 18 years. Following approval from Ethic committees, and based on our calculation, a total of 70 volunteers per group (i.e. 140 in total) will be enrolled to account for potential dropouts. All participants will be given written information regarding the project and will have to signed consent forms before enrollment. Participants will be recruited through the usual clients of the spa resort of Vichy, through health-care workers (physicians, dieticians, physiotherapist, etc.), or through advertisements. Inclusions will be realized at the CHU Clermont-Ferrand or at the thermal spa resort of Vichy.

Usual thermal spa care: All participants will benefits a usual practice thermal spa treatment combining corrections of eating disorders (and a negative energy balance of 500 kcal/day), physical activity (2h30 per day, minimum), thermal spa treatment (2h per day, minimum), and health education (1h30 per day, minimum: cooking, nutrition and physical activity classes…). Physical activity will be diverse (endurance, strength, circuit training) and personalized to the target of each participant.

Psychological interventions: Participants randomized to the intervention group will benefit from psychological interventions based on validated approaches of stress (3 x 1h30 per week i.e. 9 sessions in total). Participants will attend psychological sessions by group of less than 10 individuals. Individual meeting with the psychologist will occur at least twice: at the beginning of the residential program and at the end. Psychological interventions will include various validated approaches of work-related stress: physical [19] and psychoanalytic [20] approaches, cognitive behavioral therapy,[21] acceptance and commitment therapy,[23 31] mindfulness,[24 25] etc. Participants will have to acquire techniques in order to be autonomous and pursue at-home psychological training. The 9 psychological sessions will be the following: 1) Stress management and lack of self-confidence, 2) cognitive behavioral therapy, 3) Body-centered approach: body language, 4) Management of emotions, 5) Identity approach: concept and self-

image, 6) Cognitive approach (information processing), 7) Sophrology – relaxation, 8) Food and addictive behavior, and 9) Psychopathological approach and anxiety disorders. Each session will be constructed and validated by a psychologist specialized in the field of the session and already working in the management of obese individuals. The aim is to build a psychological program that can be easily replicated for long term used after evidence based medicine proof of success of our program.

Follow-up: After the intervention phase of the study, participants will undergo a one-year at-home follow-up with measures at 6 and 12 months.

Measurements: Each participants will perform a battery of tests (described below). Data collection will be performed 5 times as previously described (inclusion, at the start, at the end of the spa program, at 6 and 12 months), except for DXA and pQCT which will be performed at inclusion and after 12 months, cardiac remodeling and function which will be performed at inclusion and after 6 months.

Primary outcome:

Heart rate variability: Our primary outcome will be changes in HRV parameters. HRV parameters will be assessed during 26 hours with a heart rate transmitter belt simply positioned on the chest, with a 26h recording time, a beat per minute within a 25-240 range, and respiratory rate within a 3-70 range (Zephyr™ BioHarness™ BT, Zephyr Technology, Annapolis, USA). The HRV data will be examined according to the recommendations of the European Society of Cardiology and the North American Society (Task Force). HRV will be explored in time and frequency domains. [32] The methodology developed by our team will also be applied.[33] Premature beats will be visually checked and automatically discarded. In time domain, we will analyze R-R intervals, standard deviation of R-R intervals (SDNN), square root of the mean squared difference of successive R-R intervals (rMSSD), number of adjacent N-N differing by more than 50 milliseconds divided by the total number of N-N intervals (pNN50). The rMSSD and pNN50 are associated with high-frequency power (HF) and hence parasympathetic activity. In the spectral domain, we will analyze low-frequency (LF; 0.04–0.15 Hz) and high-frequency (0.15–0.4 Hz) power. LF is an index of both sympathetic and parasympathetic activity, and HF represents the

main efferent parasympathetic (vagal) activity to the sinus node. Very low frequency (VLF; 0.003–0.04 Hz) partially reflects variation within activity of the renin–angiotensin system, thermoregulatory mechanisms, and function of peripheral chemoreceptors. LF and HF will also be assessed in normalised units (nu) i.e. the relative value of each power component in proportion to the total power minus the VLF component. Thus, LFnu and HFnu were proposed to represent best sympathetic and parasympathetic activity, respectively. The LF/HF ratio, i.e. the sympathovagal balance, will also be calculated.[14]

Secondary outcomes :

Table 1 synthetize the secondary outcomes of the project.

Anthropometry and clinical parameters will be measured such as height *(m)*, body mass *(kg)*, or blood pressure (*mmHg*). Waist circumference (*cm*) was measured at mid-abdomen, midpoint between sub-costal and supra-iliac landmarks, according to the World Health Organization protocol.[34]

Body Composition: Body composition (muscle mass and fat mass) will be measured by DXA (DXA, QDR-4500A, Hologic, Inc., Waltham, MA)[35] and by impedancemeter.[36]

Biomarkers of stress and cardiovascular risk: Skin conductance will be measured using wrist band electrodes with sampling rates at 2, 4, 8, 16 and 32 Hz during the phases 1 to 3. The SC sensor (Q-Sensor®-Affectiva®, Massachusetts Institute of Technology, USA) is set on a wristband and has a 24-hour battery life when logging. In addition, it will measure wrist movements with a build in 3-Axis accelerometer.[37]

Blood flow velocity and myocardial longitudinal strain will be measured by Speckle echocardiography (Vivid Q, GE Healthcare, USA). All 2D-dimensionnal, time-motion, Doppler and 2D-strain acquisitions and measurements will be performed according to recent guidelines.[38 39] Left ventricular (LV) volumes and ejection fraction (EF) will be measured using the Simpson's biplane method. LV mass (LVM) will be calculated by the Devereux formula and indexed for height (Cornell adjustment). Pulsed-Doppler LV transmitral velocities, including early (E) and atrial (A) waves, will be obtained in the apical 4-chamber view. Tissue Doppler Imaging (TDI) measures of myocardial systolic (S'), early diastolic (E') and atrial (A') velocities will be assessed at the mitral annulus level in the apical 4- and 2-chamber views. The

E/Em ratio was used as an index of LV filling pressure.[40] Left atrium (LA) volume was assessed on apical 4- and 2 chamber views. A graduation of LV diastolic dysfunction will be obtained according to recent guidelines.[40] 2D cine-loops (frame rate >70ips) of at least 5 cycles will recorded in the short-axis views (base, md, apex), as well as in the apical 4-, 3- and 2-chambers views. 2D-strain analysis will be performed post-processing using EchoPAC 201TM software (GE Healthcare, USA). Longitudinal and circumferential strains and strain rates (SR), as well as rotations at the apex and base, will be directly obtained from the 6 segment model. Twist mechanics will be computed from apical and basal rotational data using dedicated software (Scilab, Paris, France). For each view, the 3 cardiac cycles displaying the best image quality will be selected. Blood pressure and heart rate will be continuously monitored, and the systolic meridional wall stress, an index of afterload, will be calculated. LV end-diastolic volumes will also be obtained as preload index.

Endocrine assays: Blood samples will be collected by a qualified nurse after participants have fasted overnight. Blood will be collected using a venipuncture at the brachial vein. After collection, blood will be centrifuged and aliquots will be stored *(-80°)* for subsequent analysis. Basic biology (e.g. triglycerides, cholesterol, LDLC, HDLC, HbA1c)[35] as well as all other biochemical determination (e.g., leptin,[35] ghrelin,[7 41] BDNF, [42-44] IL-1β,[35] IL-6,[35] IL-1,[35] TNFα,[35] NPY,[45] cortisol,[46 47] DHEAS[46 47]) will be assessed in the biochemistry laboratory of Clermont-Ferrand University Hospital. All analyses will be conducted by the same technician. Polymorphism of the angiotensin converting enzyme[48-51] and polymorphism of the serotonin[52-54] will be measured by blood cells, as well as telomeres length,[55] that are all linked with stress.

Complementary measures: Stress, fatigue, and sleep (Visual analogue scale of 100 mm),[10] burn out (Maslach Burn Out Inventory),[56] depression and anxiety (Hospital Anxiety and Depression Scale, STAI-Y and Hamilton scale for anxiety 7 items),[57-59] mindfulness (Freiburg Mindfulness Inventory),[22,23] coping strategies (Brief COPE Questionnaire),[60] emotions (Emotion Regulation Questionnaire),[61] perception of work (Job Content Questionnaire of Karasek),[62 63] self-efficacy (Perceived self-efficacy scale),[64] alexithymia (Toronto Alexithymia Scale),[65-67] illness perception (Brief Illness Perception Questionnaire (B-IPQ)),[68 69] metacognition (MetaCognition Questionnaire),[70] general health (General health questionnaire 36 items),[71] lifestyle (smoking, alcohol…),[10] demographics (such as marital status,

number of children, etc), nutrition (3-day self-report questionnaire with a face-to-face validation with a dietitian)[2] and physical activity (Recent Physical Activity Questionnaire (RPAQ))[72] will be obtained by questionnaires.

Bone parameters: Bone microarchitecture will be measured by Peripheral Quantitative Computed Tomography (pQCT, XCT 3000 Stratec Medizintechnik Pforzheim, Germany).[73-75] Bone mineral content *(g/cm)*, volumetric cortical and trabecular BMC *(mg/cm³)*, total area *(mm²)*, cortical and trabecular area *(mm²)* and density *(g/cm²)*, bone strength *(mm³)*, will be assessed by tomographic slices of 2 mm thickness at the distal (4%), proximal (66%) site of the non-dominant tibia and radius. Scan speed and voxel size are 20 mm/s and 0.4 mm, respectively. To assure quality of measurement, calibration checks will be performed by scanning a standard phantom with known densities, prior to each scan. Bone densitometry will be measured by Dual energy X ray Absorptiometry (DXA, QDR-4500A, Hologic, Inc., Waltham, MA). Bone mineral density (BMD, g/cm^2), bone mineral content (BMC, g), bone area (cm^2) will be determined for each participant. The DXA measurements will be taken for whole body, lumbar spine (L2-L4) and non-dominant hip (including the femoral neck, trochanteric and intertrochanteric regions). All DXA scans will be conducted by the same technician and quality assurance checks will be performed routinely. The in-vivo coefficient of variation (CV) is 0.5%.

Observance with physical activity, nutrition, and psychological techniques will be retrieved. Physical activity will be assessed with the use of RPAQ at M6 and M12.[72] Nutrition will be assessed by using three days self-report questionnaire with a face-to-face validation with a dietitian at M6 and M12.[2] The use of psychological techniques will be measured by monthly self-report questionnaires (number of use per month for each technique).

Statistical analysis: Statistical analysis will be performed using Stata software (version 13; Stata-Corp, College Station, Tex., USA). All statistical tests will be two-sided and $p<0.05$ will be considered significant. The statistics will be analyzed in intention-to-treat. After testing for normal distribution (Shapiro-Wilk test), data will be treated either by parametric or non-parametric analyses according to

statistical assumptions. Inter-groups comparisons will be performed 1) without adjustment and 2) adjusting on possible confounder's factors.

To highlight that the spa residential program will have long-term benefits (one year) on biomarkers of stress, the comparisons will be performed using Student t-test or Mann-Whitney test if assumptions of t-test are not respected (normality and homoscedasticity analyzed using Fisher-Snedecor's test). The results will be expressed as effect-size and 95% confidence interval. This primary analysis will be completed by multivariable analysis (linear regression with logarithmic transformation of dependent outcome if necessary) considering an adjustment on covariates fixed according to univariate results, clinical and epidemiological relevance (notably age, gender, baseline BMI and baseline stress levels) and observance to physical activity, nutrition, and the use of psychological techniques. The results will be expressed as regression coefficients and 95% confidence intervals.

Comparisons between groups will be performed similarly as presented previously for quantitative outcomes. Comparisons concerning categorical variables will be performed using Chi-squared or when appropriate Fischer-exact test. The results will be expressed as absolute risk differences and 95% confidence intervals. Then, the multivariable analysis will be conducted using linear and generalized linear models according to the statistical nature of dependent endpoint. The results will be expressed as regression coefficients or relative risks, and 95% confidence intervals.

Moreover, the relations between quantitative parameters will be analyzed using correlation coefficients (Pearson or Spearman according to statistical distribution). Considering the several multiple comparisons, a correction of the type I error will be applied (Sidak's correction). The comparisons of correlation coefficients (in different groups of subjects and within a single group of subjects) will be performed using Fisher's Z transformation [76] and Williams' T2 statistic [77]. Multidimensional factorial analyses will be performed to complete these statistical analyses.

Concerning the parameters collected longitudinally, mixed models will be performed to study fixed effects – group, time points evaluation and their interaction – taking into account between and within subject variability (as random-effect). For continuous endpoints, the normality of residuals will be assessed using the Shapiro-Wilk test.

In addition to previous analyses, to study differences during follow-up (end of the spa program, 6 and 12 months), these analyses will be completed using ANCOVA considering values at baseline.[78] Normality of residuals will be verified.

A sensitivity analysis will be realized to study the statistical nature of missing data (at random or not) and then, to apply the most appropriate imputation data method (multiple imputation data, last observation carried out). Baseline characteristics of participants who will have a complete follow-up and those who will be lost to follow-up will be compared with statistical tests aforementioned.

Radiation exposure and harms

Both DXA and pQCT provide measures of body composition and bone properties by exposing participants to low level radiation: 0.0056 mSv from DXA scans *(whole body, lumbar and hip)* and 0.0014 mSv from the pQCT scans *(tibia and radius measures)* [79]. Over the duration of each study, the effective dose of 0.014 mSv will be administered.

A Harms section was not considered in the protocol, but this kind of intervention was considered as very low risk by the ethics committee.

Confidentiality and blind assessments

Despite the participants and care providers will not be blinded to the participants' allocation group, in order to reduce the level of bias, assessors for most outcomes will be blinded to the assignment group of each participant, such as for HRV, biological measures, or bone parameters. All outcome data will remain blinded until the end of the study. Patient's data will be deidentified and all data will be treated anonymously.

Patient and public involvement

The Thermal spa center of Vichy in collaboration with the Preventive and Occupational Medicine Department of the University Hospital of Clermont-Ferrand identified and addressed the following priorities: prevention, obesity, weight loss, stress, cardiovascular morbidity. We are grateful for the opinion of volunteers and professionals from the Vichy Spa Center on the psychological intervention.

Conferences and meetings with participants will be organised in order to provide them feedback from this research.

Ethical considerations and dissemination

The ObesiStress protocol complies with the Ethics guidelines for Clinical Research and has been approved by the Ethic Committee (CPP Sud-Est VI, Clermont-Ferrand - ANSM: 2016-A01774-47), the protocol has also been registered in Clinical Trial NCT 03578757. In accordance with Ethical considerations, the chief investigator is responsible of ensuring that participants understand potential risks and benefits of taking part in the study. Moreover, the chief investigator is responsible for obtaining writing consent from participants. The results will be disseminated at several research conferences and published articles in peer-reviewed journals.

Discussion

The ObesiStress protocol was designed to provide a better understanding of the effect of a spa residential program combined with a stress management program on the improvement of heart rate variability in the treatment of obesity. The creation of a new thermal program would allow new innovative approaches for stress management in obesity. The long-term success of lifestyle interventions such as those proposed in prevention of obesity is the observance to the treatment (nutrition, physical activity, psychology).[80] We previously demonstrated that a spa program may play a major role in sustainable lifestyle changes.[35] Because of the stress management program and because the participants will be accompanied by health-care professionals, the observance to treatment during a one-year follow-up could be more efficient. In order to avoid generalizability of our expected results, we will pay a particular attention at the demographics of included participants (particularly between participants recruited from the "usual clients of the spa", and other participants). Secondary and sensitivity analyses will take into account provenance of participants.

Current study status

The status of the trial is ongoing recruitment.

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Competing interests

The authors declare that they have no competing interests.

Author's contributions

FD contributed to the study design. FD was responsible for ethics committee. FD and AV will coordinate the recruitment of patients. DC, EC and PD will be responsible for bone measures. AA will be responsible for liver-related factors. DP and SH will be responsible for psychosocial analysis. LM and MM will be responsible for mindfulness, psychological education and cognitive-compartmental therapy analysis. GV will be responsible for the analysis of memory-related data. PO, OI and GB will be responsible for cardiovascular measures. YB, NF and MMD will be responsible for nutritional measures and analysis. DC, EC and PD will be responsible for physical activity analysis. AV will be responsible for the collection of psychological factors. BP is responsible for the statistical analysis. EC was responsible for the clinical trial and wrote the first draft of this manuscript. All authors read, contributed towards and approved of the final manuscript.

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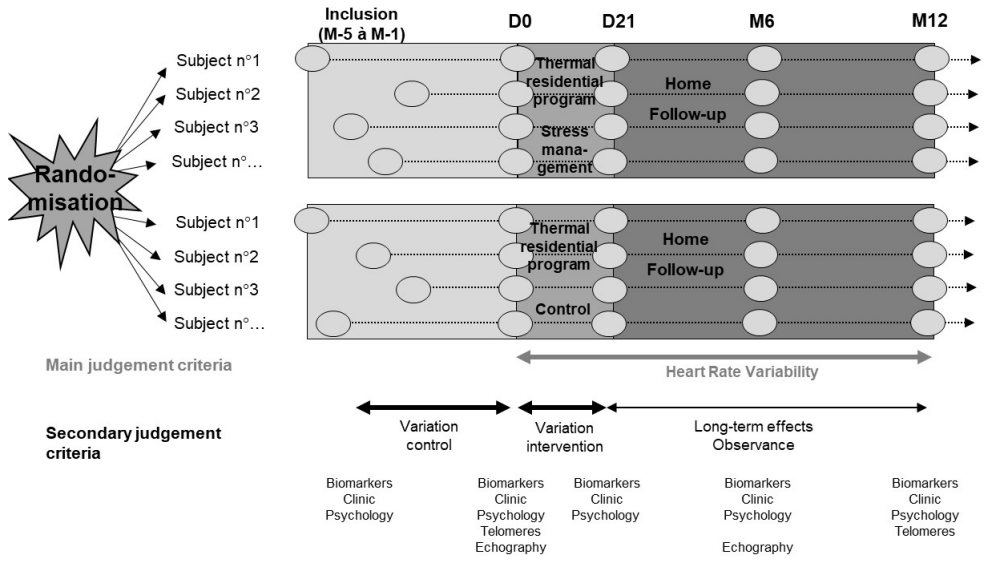
Table 1. Outcomes

Variables	Type of Measures	Modalities to measure	References
Biomarkers of stress and cardiovascular risk	Heart rate variability	Holter	81
	Skin conductance	Wristband electrodes – Movisens	37
	Blood flow velocity	Laser speckle contrast imaging (LSCI)	82 83
	Myocardial longitudinal strain	Speckle tracking echocardiography (STE)	38 39
Genetic polymorphisms	Polymorphism of the angiotensin converting enzyme	Blood cells	48-51
	Polymorphism of the serotonin	Blood cells	52-54
Demographics* (* Adjustment variables)	Age, gender, qualification, personal work status, ethnicity, life and occupational events	Questionnaire	10
Clinical measurements		Height, weight, blood pressure, heart rate, Waist circumference	
Body composition	Muscle mass, fat mass, bone structure	Impedancemeter	36
		Densitometry X-ray absorption	35
		Peripheral quantitative computed tomography (pQCT)	73-75
		Quantitative ultrasounds (QUS)	84
Psychology and quality of life	Depression	HAD 7 items	57
	Anxiety	HAD 7 items Hamilton scale for anxiety 7 items	57
		State and Trait Anxiety Inventory (STAI)	58 59
	General health	General health questionnaire SF-36 36 items	71
	Quality of life	Brief Multidimensional Life Satisfaction Scale 11 items	85
	Stress, fatigue, sleep	Visual analog scale of 100 mm	10
	Burn-out	Maslach Burn-Out Inventory	56
	Mindfulness	Mindfulness Fribourg Mindfulness Inventory	22,23
	Coping	Brief COPE questionnaire (BCQ)	60
	Emotions	Emotion Regulation Questionnaire (ERQ)	61
	Perception of work	Job Content Questionnaire of Karasek	63
	Self-efficacy	Perceived self-efficacy scale	64
	Alexithymia	Toronto Alexithymia Scale (TAS-20)	65-67
	Illness Perception	Brief Illness Perception Questionnaire (B-IPQ)	68 69
	Metacognition	MetaCognition Questionnaire (MCQ-30)	70
Personal resources	Trait perception of workplace stress	Inner Correspondence/Peaceful Harmony with practices (ICPH) 17 items	86
Lifestyle	Smoking, alcohol, coffee, food intake…	Questionnaires	10
	Physical activity	Recent Physical Activity Questionnaire (RPAQ)	72
Alloplastic load	HbA1c, HDLc and LDL-cholesterol, TG	EDTA tube	35

	Cortisol	Dry tube, serum isolation and deep-freezing	46 47
	DHEAS	Dry tube, serum isolation and deep-freezing	46 47
	Leptin	Dry tube, serum isolation and deep-freezing	35
	Ghrelin	Dry tube, serum isolation and deep-freezing	7 41
	BDNF	Dry tube, serum isolation and deep-freezing	42-44
	Pro-inflammatory Cytokines: IL-1β, IL-6, IL-1, TNFα	Dry tube, serum isolation and deep-freezing	35
	NPY	Dry tube, serum isolation and deep-freezing	45
	Telomeres length	Blood; analyses by southern blot or PCR	55

Figure Title: ObestiStress protocol

Figure Abbreviation: M months, D days



Title: The ObesiStress protocol
M months, D day

105x59mm (300 x 300 DPI)

Reporting checklist for protocol of a clinical trial.

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Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	Page 2
Funding	#4	Sources and types of financial, material, and other support	Page 14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Pages 1-2;14
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	Page 2

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Page 14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	
Objectives	#7	Specific objectives or hypotheses	Page 5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	Page 6
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Pages 7-8

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Pages 8-11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Pages 6, 8, Figure 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Pages 6-7
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 7
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 6
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Pages 8-11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Pages 11-12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Pages 11-12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple	Pages 11-12

		imputation)	
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	Page 24
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	
Declaration of	#28	Financial and other competing interests for principal	Page 14

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 13
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

BMJ Open

ObesiStress: Stress Management in Obesity during a thermal spa residential programme – a protocol for a randomised controlled trial study

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Complete List of Authors:	DUTHEIL, Frédéric; Occupational Medicine, University Hospital CHU G. Montpied; Australian Catholic University, Faculty of Health, School of Exercise Science CHAPLAIS, Elodie; Australian Catholic University, School of Exercise Science; University of Lorraine; DevAH Laboratory (EA 3450), Exercise Sciences VILMANT, Audrey; Occupational Medicine, University Hospital CHU G. Montpied COURTEIX, Daniel; Laboratoire AME2P - Université Clermont Auvergne; Australian Catholic University, School of Exercise Science DUCHE, Pascale; AME2P Labratory - Université Clermont Auvergne ABERGEL, Armand; Université Clermont Auvergne, CNRS, UMR 6284, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Hepatology Gastroenterology PFABIGAN, Daniela M. ; School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behavior and Mental Health, Peking University HAN, Shihui; School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behavior and Mental Health, Peking University MONDILLON, Laurie; University Clermont Auvergne, and LAPSCO-CNRS, Psychology VALLET, Guillaume T.; Université Clermont Auvergne, CNRS, LaPSCo, Physiological and Psychosocial Stress Mermillod, Martial; Centre Hospitalier Universitaire Grenoble Alpes BOUDET, Gil; Occupational Medicine, University Hospital CHU G. Montpied Obert, Philippe; University of Avignon, Laboratory of Pharm-Ecology Cardiovascular EA4278 Izem, Omar; Laboratory of Cardiovascular Pharm-ecology (LaPEC EA4278), Avignon University MIOLANNE-DEBOUIT, Magalie; University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition Farigon, Nicolas; University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition Pereira, Bruno; University Hospital CHU Clermont-Ferrand, Boirie, Yves; Université Clermont Auvergne, INRA, CRNH, University Hospital of Clermont–Ferrand, CHU Clermont–Ferrand, Unit of Human Nutrition

Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Nutrition and metabolism, Sports and exercise medicine, Public health
Keywords:	obesity, stress, prevention, heart rate variability, spa bath

ObesiStress: Stress Management in Obesity during a thermal spa residential programme – a protocol for a randomised controlled trial study

Frédéric DUTHEIL[1,2], Elodie CHAPLAIS[2,4,5,15], Audrey Vilmant[1], Daniel COURTEIX[2,3], Pascale DUCHE[3], Armand ABERGEL[6] , Daniela M. PFABIGAN[7], Shihui HAN[7], Laurie MONDILLON[8], Guillaume T. VALLET[8], Martial MERMILLOD[9,10], Gil BOUDET[1], Philippe OBERT[2,11], Omar IZEM[11], Magalie MIOLANNE-DEBOUIT[12], Nicolas FARIGON[12], Bruno PEREIRA[13], Yves BOIRIE[14]

Authors' affiliations:

[1] Université Clermont Auvergne, CNRS, LaPSCo, Physiological and Psychosocial Stress, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Preventive and Occupational Medicine, WittyFit, Clermont-Ferrand, France

[2] Australian Catholic University, Faculty of Health, School of Exercise Science, Melbourne, Victoria 3065, Australia

[3] Université Clermont Auvergne, Laboratory of Metabolic Adaptations to Exercise under Physiological and Pathological Conditions (AME2P - EA 3533), Clermont-Ferrand, France

[4] Australian Catholic University, Faculty of Health Science, School of Exercise Science, Strathfield, NSW 2135, Australia

[5] University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Biostatistics Unit, Clinical research and Innovation Department (DRCI), Clermont-Ferrand, France

[6] Université Clermont Auvergne, CNRS, UMR 6284, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Hepatology Gastroenterology, Clermont-Ferrand, France

[7] School of Psychological and Cognitive Sciences, PKU-IDG/McGovern Institute for Brain Research, Beijing Key Laboratory of Behaviour and Mental Health, Peking University, Beijing, China

[8] Clermont Auvergne University, CNRS, LaPSCo, Physiological and Psychosocial Stress, Clermont-Ferrand, France

[9] LNPC, CNRS, Université Grenoble Alpes, Université Savoie Mont Blanc, Grenoble, France

[10] Institut Universitaire de France, Paris, France

[11] Laboratory of Cardiovascular Pharm-ecology (LaPEC EA4278), Avignon University, Avignon, France

[12] University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Unit of Human Nutrition, Clermont-Ferrand, France.

[13] University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Biostatistics Unit, Clinical research and Innovation Department (DRCI), Clermont-Ferrand, France

[14] Université Clermont Auvergne, INRA, CRNH, University Hospital of Clermont-Ferrand, CHU Clermont-Ferrand, Unit of Human Nutrition, Clermont-Ferrand, France.

[15] Université de Lorraine, Laboratory "Development, Adaptation and Disability" (DevAH - EA 3450), Nancy, France

Corresponding author – CHAPLAIS Elodie (PhD), University of Lorraine, Laboratory "Development, Adaptation and Disability" (EA 3450), Faculty of Sports Sciences and Faculty of Medicine, 9 avenue de la Forêt de Haye, CS 50184, F-54505 Vandœuvre-lès-Nancy, France; elodie.chaplais@acu.edu.au

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Abstract

Introduction: Stress and obesity are two public health issues. The relationship between obesity and stress is biological through the actions of stress on the major hormones that regulate appetite (leptin, ghrelin). Many spa resorts in France are specialised in the treatment of obesity, but no thermal spa currently proposes a specific programme to manage stress in obesity. The ObesiStress protocol has been designed to offer a new residential stress management programme. This thermal spa treatment of obesity implement stress management strategies as suggested by International recommendations.

Methods and analysis: 140 overweight or obese participants with a Body Mass Index (BMI) >25 kg.m-2 and aged over 18 years will be recruited. Participants will be randomised into two groups: a control group of usual practice (restrictive diet, physical activity and thermal spa treatment) and an intervention group with stress management in addition to the usual practice. In the present protocol, parameters will be measured on five occasions (at inclusion, at the beginning of the spa (day 0), at the end of the spa (day 21), and at 6 and 12 months). The study will assess the participants' heart rate variability, cardiac remodelling and function, electrodermal activity, blood markers, anthropometric profile, body composition, psychology and quality of life via the use of questionnaires and bone parameters.

Ethics and dissemination: The ObesiStress protocol complies with the ethics guidelines for Clinical Research and has been approved by the ethics committee (CPP Sud-Est VI, Clermont-Ferrand - ANSM: 2016-A01774-47). This study aims to highlight the efficacy of a 21-day thermal spa residential programme of stress management in obesity through objective measurements of well-being and cardiovascular morbidity. Results will be disseminated during several research conferences and articles published in peer-reviewed journals.

Trial Registration: NCT 03578757

Strengths and limitations of this study:

- Reliability and validity of a 21-day specific thermal spa residential programme on stress management in the treatment of obesity
- Better comprehension of the psychology and psychological mechanisms involved in stress management in obesity.
- A 12-month mixed gender longitudinal study
- Inability to accurately account for the long-term cost-effective benefits of the study

Introduction

Stress and obesity are two public health issues.[1 2] Stress can lead to obesity, is a major stress factor,[3] via inappropriate eating behaviours [4]. Furthermore, stressed people are also those who have the greatest difficulty losing weight.[5] The relationship between obesity and stress is biological through the action of stress on the major hormones which regulate appetite (leptin, ghrelin).[6 7] The relationship between obesity and stress is so strong that proposals for international recommendations suggest the implementation of stress management programmes in obesity for sustainable weight loss.[8]

Among the multiple physical and psychological consequences of stress and obesity, increased mortality and cardiovascular morbidity seem to be the main concern.[9] Stress and obesity alter the functioning of the autonomic nervous system. [10-12] A deregulation of the sympatho-vagal balance is a major factor of morbidity cardiovascular mortality.[13] Conveniently, the sympatho-vagal balance can be measured easily and without pain using heart rate variability (HRV), which is a biomarker of both stress and morbidity/mortality.[10 14 15] Stress and obesity also cause arterial ischaemic pathology [16] through complex mechanisms involving changes in endothelial and arterial atherosclerosis.[16] These microvascular changes are linked to systemic inflammation caused by stress[17] and obesity.[18]

The thermal spa resort in Vichy, as well as other spa centres in France, already possesses expertise in obesity treatment through physical activity, diet, and hydrotherapy. However, no spa resort has ever proposed the inclusion of a stress-management programme in obesity treatment. Non-pharmacological stress management can be achieved through psychological interventions (i.e. physical [19] and psychoanalytical approaches [20], cognitive behavioural therapy,[21] acceptance and commitment therapy,[22 23] or mindfulness [24 25]), physical activity,[26 27] and the improvement of eating disorders induced by stress.[28]

The benefits of physical activity on physical and mental health are indisputable, at any age, and with any activity.[29]

The main hypothesis of this project is that a thermal spa residential programme (21 days) of stress management in obesity will demonstrate its efficacy through objective measurements of well-being and cardiovascular morbidity via a randomised controlled design that compares a group with stress management and a group without stress management (both groups will benefit from the same spa treatments, physical activity, and diet).

Objectives

The main objective will be to assess the ability of a 21-day residential spa programme of stress management in the treatment of obesity to increase heart rate variability; a biomarker of both stress and morbidity/mortality.

Secondary outcomes will be (1) to demonstrate an improvement in stress- and obesity-related variables following the short residential spa programme, (2) to study the influence of genetic polymorphisms on stress, obesity, and on the response to our stress management programme, (3) to examine the relationship between stress- and obesity-related variables, (4) to propose a salient biomarker or a salient composite index of biomarkers of stress in obesity and (5) to study the effect of adherence to the programme during follow-up on stress- and obesity-related variables.

Methods

The TIDieR checklist can be found as supplementary file (S1).

Protocol design: This one-year randomised controlled study with repeated measurements at five time points (inclusion, at the start and the end of the spa programme, at 6 and 12 months) will allow us to understand the effect of a 21-day residential spa programme of stress management in the treatment of obesity through the measurement of well-being and cardiovascular morbidity.

For this study, two randomised groups of overweight or obese participants will be compared: one will receive the usual 21-day thermal spa residential programme while the other will receive the 21-day thermal spa residential programme plus a psychological intervention (Figure1).

Randomisation

Randomisation will be stratified by BMI category (25-30, 30-35, >35), sex, and levels of stress (visual analog scale of stress <50, between 50 and 80, >80), using a minimisation approach. A permuted-block randomisation (i.e. random block sizes) will be conducted using a computer-generated random allocation (Stata software, version 13, StataCorp, College Station, USA), with a 1:1 allocation ratio,

ensuring the complete randomness of the assignment of a participant to each randomised group. To guarantee the concealment of the allocation, the participants will be randomised after they have clearly met the inclusion criteria and have provided written consent (supplementary file – S2).

Selection criteria:

Inclusion criteria: Volunteers will be overweight or obese participants aged over 18 years who wish to follow a thermal spa residential programme for the treatment of obesity. We will also promote the study through advertisements in local newspapers and on the radio. Volunteers will be screened by telephone interview or directly by spa physicians. A participant's weight must have been stable over the last three months, with no uncontrolled cardiac, hepatic, renal or endocrine diseases.[2] Stress at baseline will not be an inclusion criteria but an explanatory/independent variable. In compliance with Human Ethics guidelines, participants will have to be covered by social health insurance and will have to sign consent forms.

Exclusion criteria: Volunteers participating in the study will be excluded if major treatment and/or protocol deviations are observed.[14] Drugs and medical conditions that significantly affect the primary outcome (heart rate variability) will also be exclusion criteria (e.g. alpha or beta blockers, arrhythmia or conduction disorders such as bundle branch block, atrioventricular heart block).[30] Bariatric surgery is also an exclusion criterion.

Power analysis: The rationale for the sample size calculation is based on HRV, which is a biomarker of both stress and morbidity/mortality.[10 14 15] Specifically, within the multiple parameters of HRV, we considered the log Low-Frequency/High-Frequency (LF/HF) for the sample size calculation because it is the parameter that traditionally represents sympathovagal balance (see description of LF/HF below in the description of the primary outcome).[10 14] A log LF/HF with low values is associated with a good adaptation of the autonomic nervous system. Based on our results from a pilot study (data not published),[2] we hope to highlight an absolute difference of 12% between the groups with regard to the decrease of log LF/HF at one year after the stress management programme. For a standard deviation of 20%, the expected size will be around 0.60. For a two-sided type I error of 5%, we will need to include

59 participants per group to achieve a statistical power of 90%. Finally, the recruitment of 70 patients per arm is proposed in order to take into account lost to follow-up.

Participants: As previously stated, participants engaged in this protocol will be mixed gender overweight or obese volunteers aged over 18 years. Following approval from the ethics committee, and based on our calculation, a total of 70 volunteers will be enrolled per group (i.e. a total of 140 participants) to account for potential dropouts. All participants will be given written information regarding the project and will have to sign consent forms before enrolment. Participants will be recruited from the usual clients at the spa resort in Vichy, through healthcare workers (physicians, dietitians, physiotherapist, etc.), or through advertisements. Inclusions will be carried out at the University Hospital in Clermont-Ferrand or at the thermal spa resort in Vichy.

Usual thermal spa care: All participants will undergo the usual thermal spa treatment that combines the correction of eating disorders (and a negative energy balance of 500 kcal/day), physical activity (2h30 per day, minimum), thermal spa treatment (2h per day, minimum), and health education (1h30 per day, minimum: cooking, nutrition and physical activity classes, etc.). Physical activity will be diverse (endurance, strength, circuit training) and personalised for each participant.

Psychological interventions: Participants randomised to the intervention group will benefit from psychological interventions based on validated approaches to stress (3 x 1h30 per week, i.e. 9 sessions in total). Participants will attend psychological sessions in groups of fewer than 10 individuals. Individual meetings with the psychologist will occur at least twice: at the beginning of the residential programme and at the end. Psychological interventions will include various validated approaches to work-related stress: physical [19] and psychoanalytic [20] approaches, cognitive behavioural therapy,[21] acceptance and commitment therapy,[23 31] mindfulness,[24 25] etc. Participants will have to acquire techniques in order to become autonomous and pursue at-home psychological training. The 9 psychological sessions will be the following: 1) stress management and lack of self-confidence, 2) cognitive behavioural therapy, 3) body-centred approach: body language, 4) management of emotions,

5) identity approach: concept and self-image, 6) cognitive approach (information processing), 7) sophrology – relaxation, 8) food and addictive behaviour, and 9) psychopathological approach and anxiety disorders. Each session will be constructed and validated by a psychologist specialised in the session's field and already working in the management of obese individuals. The aim is to build a psychological programme that can be easily replicated for long-term use after evidence based proof of success.

Follow-up: After the intervention phase of the study, participants will undergo a one-year at-home follow-up with measurements at 6 and 12 months.

Measurements: Each participant will perform a battery of tests (described below). As previously described, data collection will be performed 5 times (at inclusion, at the start and the end of the spa programme, and at 6 and 12 months), with the exception of DXA and pQCT, which will be performed at inclusion and after 12 months, and cardiac remodelling and function, which will be performed at inclusion and after 6 months.

Primary outcome:

Heart rate variability: Our primary outcome will be changes in HRV parameters. HRV parameters will be assessed over 26 hours with a heart rate transmitter belt simply positioned on the chest, with a 26h recording time, a beat per minute range of 25-240, and a respiratory rate range of 3-70 (Zephyr™ BioHarness™ BT, Zephyr Technology, Annapolis, USA). The HRV data will be examined according to the recommendations of the European Society of Cardiology and the North American Society (Task Force). HRV will be explored in two domains: time and frequency. [32] The methodology developed by our team will also be applied.[33] Premature beats will be visually checked and automatically discarded. In the time domain, we will analyse R-R intervals, the standard deviation of the R-R intervals (SDNN), the square root of the mean squared difference of successive R-R intervals (rMSSD), and the number of adjacent N-N differing by more than 50 milliseconds divided by the total number of N-N intervals (pNN50). The rMSSD and pNN50 are associated with high-frequency power (HF) and hence

parasympathetic activity. In the spectral domain, we will analyse low-frequency (LF; 0.04–0.15 Hz) and high-frequency (0.15–0.4 Hz) power. LF is an index of both sympathetic and parasympathetic activity, and HF represents the main efferent parasympathetic (vagal) activity to the sinus node. Very low frequency (VLF; 0.003–0.04 Hz) partially reflects variations in the activity of the renin – angiotensin system, thermoregulatory mechanisms, and the function of peripheral chemoreceptors. LF and HF will also be assessed in normalised units (nu), i.e. the relative value of each power component in proportion to the total power minus the VLF component. Thus, LFnu and HFnu are suggested to represent the best sympathetic and parasympathetic activity, respectively. The LF/HF ratio, i.e. the sympathovagal balance, will also be calculated.[14]

Secondary outcomes:

Table 1 summarises the secondary outcomes of the project.

Anthropometry and clinical parameters will be measured, including height *(m)*, body mass *(kg)*, or blood pressure (*mmHg*). Waist circumference (*cm*) will be measured at mid-abdomen, i.e. the midpoint between the subcostal and supra-iliac landmarks, in accordance with the World Health Organization protocol.[34]

Body Composition: Body composition (muscle mass and fat mass) will be measured by DXA (DXA, QDR-4500A, Hologic, Inc., Waltham, MA)[35] and by an impedance meter.[36]

Biomarkers of stress and cardiovascular risk: Skin conductance will be measured using wrist band electrodes with sampling rates at 2 Hz, 4 Hz, 8 Hz, 16 Hz and 32 Hz during phases 1 to 3. The SC sensor (Q-Sensor®-Affectiva®, Massachusetts Institute of Technology, USA) is set on a wristband and has a 24-hour battery life when recording. In addition, it will measure wrist movements with a built-in 3-axis accelerometer.[37]

Blood flow velocity and myocardial longitudinal strain will be measured by speckle echocardiography (Vivid Q, GE Healthcare, USA). All 2-dimensional, time motion, Doppler and 2D-strain acquisitions and measurements will be performed according to recent guidelines.[38 39] Left ventricular (LV) volumes and ejection fractions (EF) will be measured using the Simpson biplane method. LV mass (LVM) will

be calculated with the Devereux formula and indexed for height (Cornell adjustment). Pulsed-Doppler LV transmitral velocities, including early (E) and atrial (A) waves, will be obtained in the apical 4-chamber view. Tissue Doppler Imaging (TDI) measurements of myocardial systolic (S'), early diastolic (E') and atrial (A') velocities will be assessed at the mitral annulus level in the apical 4- and 2-chamber views. The E/Em ratio will be used as an index of LV filling pressure.[40] Left atrium (LA) volume will be assessed on apical 4- and 2 chamber views. A graduation of LV diastolic dysfunction will be obtained according to recent guidelines.[40] 2D cine-loops (frame rate >70 ips) of at least 5 cycles will be recorded in the short-axis views (base, md, apex), as well as in the apical 4-, 3- and 2-chamber views. 2D-strain analysis will be performed post-processing using EchoPAC 201 TM software (GE Healthcare, USA). Longitudinal and circumferential strains and strain rates (SR), as well as rotations at the apex and base, will be directly obtained from the 6-segment model. Twist mechanics will be computed from apical and basal rotational data using dedicated software (Scilab, Paris, France). For each view, the 3 cardiac cycles displaying the best image quality will be selected. Blood pressure and heart rate will be continuously monitored, and the systolic meridional wall stress, an index of afterload, will be calculated. LV end-diastolic volumes will also be obtained as preload indices.

Endocrine assays: Blood samples will be collected by a qualified nurse after participants have fasted overnight. Blood will be collected using a venipuncture of the brachial vein. After collection, blood will be centrifuged and aliquots will be stored *(-80 °)* for subsequent analysis. Basic biology (e.g. triglycerides, cholesterol, LDLC, HDLC, HbA1c)[35], as well as all other biochemical determinants (e.g. leptin,[35] ghrelin,[7 41] BDNF, [42-44] IL-1β,[35] IL-6,[35] IL-1,[35] TNFα,[35] NPY,[45] cortisol,[46 47] DHEAS[46 47]), will be assessed in the biochemistry laboratory at the University Hospital in Clermont-Ferrand. All analyses will be conducted by the same technician. Polymorphism of the angiotensin converting enzyme[48-51] and polymorphism of the serotonin[52-54], as well as telomere lengths,[55] will be measured via blood cells, all of which are linked with stress.

Complementary measurements: Stress, fatigue, and sleep (visual analogue scale of 100 mm),[10] burnout (Maslach Burn Out Inventory),[56] depression and anxiety (Hospital Anxiety and Depression Scale, STAI-Y and the 7-item Hamilton scale for anxiety),[57-59] mindfulness (Freiburg Mindfulness Inventory),[22,23] coping strategies (Brief COPE Questionnaire),[60] emotions (Emotion Regulation Questionnaire),[61]

perception of work (Karasek's Job Content Questionnaire),[62 63] self-efficacy (perceived self-efficacy scale),[64] alexithymia (Toronto Alexithymia Scale),[65-67] illness perception (Brief Illness Perception Questionnaire (B-IPQ)),[68 69] metacognition (MetaCognition Questionnaire),[70] general health (36-item General health questionnaire),[71] lifestyle (smoking, alcohol, etc.),[10] demographics (such as marital status, number of children, etc.), nutrition (3-day self-report questionnaire with a face-to-face validation with a dietitian)[2] and physical activity (Recent Physical Activity Questionnaire (RPAQ))[72] will be obtained through questionnaires.

Bone parameters: Bone microarchitecture will be measured by Peripheral Quantitative Computed Tomography (pQCT, XCT 3000 Stratec Medizintechnik Pforzheim, Germany).[73-75] Bone mineral content *(g/cm)*, volumetric cortical and trabecular BMC *(mg/cm³)*, total area *(mm²)*, cortical and trabecular area *(mm²)* and density *(g/cm²)*, bone strength *(mm³)*, will be assessed by 2 mm thick tomographic slices at the distal (4%) and proximal (66%) site of the non-dominant tibia and radius. Scan speed and voxel size will be 20 mm/s and 0.4 mm, respectively. To ensure the quality of the measurements, calibration checks will be performed by scanning a standard phantom with known densities prior to each scan. Bone densitometry will be measured by Dual energy X-ray Absorptiometry (DXA, QDR-4500A, Hologic, Inc., Waltham, MA). Bone mineral density (BMD, g/cm ^{2}), bone mineral content (BMC, g), and bone area (cm ^{2}) will be determined for each participant. The DXA measurements will be taken for whole body, lumbar spine (L2-L4) and non-dominant hip (including the femoral neck, trochanteric and intertrochanteric regions). All DXA scans will be conducted by the same technician and quality assurance checks will be routinely performed. The in-vivo coefficient of variation (CV) is 0.5%.

Adherence to the physical activity, nutrition, and psychological techniques will be retrieved. Physical activity will be assessed using the RPAQ at M6 and M12.[72] Nutrition will be assessed at M6 and M12 through a three-day self-report questionnaire with a face-to-face validation with a dietitian.[2] The use of psychological techniques will be measured by monthly self-report questionnaires (number of times each technique was used per month).

Statistical analysis: Statistical analysis will be performed using the Stata software (version 13; Stata-Corp, College Station, Tex., USA). All statistical tests will be two-sided and $p<0.05$ will be considered significant. The data will be analysed as intention-to-treat. After testing for normal distribution (Shapiro-Wilk test), the data will be treated either by parametric or nonparametric analysis according to statistical assumptions. Inter-group comparisons will be performed 1) without adjustment and 2) adjusting on possible confounding factors.

To highlight that the spa residential programme will have long-term benefits (one year) on the biomarkers of stress, the comparisons will be performed using the Student t-test or the Mann-Whitney test if the t-test assumptions are not respected (normality and homoscedasticity analysed using the Fisher-Snedecor test). The results will be expressed as effect size and 95% confidence intervals. This primary analysis will be completed by multivariable analyses (linear regression with logarithmic transformation of dependent outcomes if necessary) considering an adjustment on covariates fixed according to univariate results, clinical and epidemiological relevance (notably age, gender, baseline BMI and baseline stress levels) and adherence to physical activity, nutrition, and the use of psychological techniques. The results will be expressed as regression coefficients and 95% confidence intervals.

Comparisons between groups will be performed in a similar way as presented previously for quantitative outcomes. Comparisons concerning categorical variables will be performed using Chi-squared or, when appropriate, the Fischer-exact test. The results will be expressed as absolute risk differences and 95% confidence intervals. The multivariable analysis will then be conducted using linear and generalised linear models according to the statistical nature of the dependent endpoint. The results will be expressed as regression coefficients or relative risks and 95% confidence intervals.

Moreover, the relationships between the quantitative parameters will be analysed using correlation coefficients (Pearson or Spearman depending on the statistical distribution). Considering the several multiple comparisons, a correction of the type I error will be applied (Sidak's correction). The comparisons of the correlation coefficients (in different groups of subjects and within a single group of subjects) will be performed using a Fisher's Z transformation [76] and Williams' T2 statistic [77]. Multidimensional factorial analyses will be performed to complete these statistical analyses.

Concerning the longitudinally collected parameters, mixed models will be performed to study fixed effects (group, time point evaluations and their interactions) , taking into account the between and within subject variability (as random effect). For continuous endpoints, the normality of the residuals will be assessed using the Shapiro-Wilk test.

In addition, these analyses will be completed using ANCOVA with the baseline values to study the differences during follow-up (end of the spa programme, 6 and 12 months).[78] Normality of residuals will be verified.

A sensitivity analysis will be carried out to study the statistical nature of missing data (random or not) and then to apply the most appropriate imputation data method (multiple imputation data, last observation carried out). The baseline characteristics of participants with a complete follow-up and those lost to follow-up will be compared with the aforementioned statistical tests.

Radiation Exposure and Harm

Both DXA and pQCT provide measurements of the body composition and bone properties by exposing participants to low-level radiation: 0.0056 mSv from DXA scans *(whole body, lumbar and hip)* and 0.0014 mSv from the pQCT scans *(tibia and radius measurements)* [79]. Over the duration of each study, the effective administered dose will be 0.014 mSv.

A Harms section was not considered in the protocol, but this kind of intervention was considered to be very low risk by the ethics committee.

Confidentiality and blind assessments

The participants and care providers will not be blinded to the participants' randomisation group. However, in order to reduce the level of bias, the assessors for most outcomes will be blinded to each participant's assigned group, e.g. for HRV, biological measures, or bone parameters. All outcome data will remain blinded until the end of the study. Patient's data will be de-identified and all data will be treated anonymously.

Patient and Public Involvement

The thermal spa centre in Vichy, in collaboration with the Preventive and Occupational Medicine Department of the University Hospital in Clermont-Ferrand, have identified and addressed the following priorities: prevention, obesity, weight loss, stress, cardiovascular morbidity. We are grateful for the opinion of the volunteers and professionals at the Vichy Spa Centre concerning the psychological intervention. Conferences and meetings with participants will be organised in order to provide feedback from this research.

Ethical Considerations and Dissemination

The ObesiStress protocol complies with the ethics guidelines for Clinical Research and has been approved by the ethic committee (Comités de Protection des Personnes, Sud-Est VI, Clermont-Ferrand – National Agency for Medical Security: 2016-A01774-47), the protocol has also been registered on clinicaltrials.gov (NCT 03578757). In accordance with ethical considerations, the chief investigator is responsible for ensuring that participants understand the potential risks and benefits of taking part in the study. Moreover, the chief investigator is responsible for obtaining written consent from the participants. The results will be disseminated at several research conferences and in articles published in peer-reviewed journals.

Discussion

The ObesiStress protocol has been designed to provide a better understanding of the effect of a spa residential programme combined with a stress management programme on the improvement of heart rate variability in the treatment of obesity. The creation of a new thermal programme would allow new innovative approaches for stress management in obesity. The long-term success of lifestyle interventions such as those proposed in the prevention of obesity is adherence to the treatment (nutrition, physical activity, psychology).[80] We previously demonstrated that a spa programme may play a major role in sustainable lifestyle changes.[35] Due to the stress management programme and because the participants will be accompanied by healthcare professionals, the adherence to treatment during a one-year follow-up could be more efficient. In order to avoid any generalisability of our expected results, we will pay particular attention at the demographics of the participants included (particularly between participants

recruited from the “usual spa clients" and other participants). Secondary and sensitivity analyses will take into account where the participants were recruited.

Current study status

The trial is currently recruiting participants.

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Competing interests

The authors declare that they have no competing interests.

Author’s contributions

FD contributed to the study design. FD was responsible for the submission of the protocol to the ethics committee. FD and AV will coordinate the recruitment of patients. DC, EC and PD will be responsible for bone measurements. AA will be responsible for liver-related factors. DP and SH will be responsible for psychosocial analysis. LM and MM will be responsible for mindfulness, psychological education and cognitive-compartmental therapy analysis. GV will be responsible for the analysis of memory-related data. PO, OI and GB will be responsible for cardiovascular measurements. YB, NF and MMD will be responsible for nutritional measurements and analysis. DC, EC and PD will be responsible for physical activity analysis. AV will be responsible for the collection of psychological factors. BP is responsible for the statistical analysis. EC is responsible for the clinical trial and wrote the first draft of this manuscript. All authors read, contributed towards and approved the final manuscript.

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Table 1. Outcomes

Variables	Type of Measurements	Modalities of measurement	References
Stress and cardiovascular risk biomarkers	Heart rate variability	Holter	81
	Skin conductance	Wristband electrodes – Movisens	37
	Blood flow velocity	Laser speckle contrast imaging (LSCI)	82 83
	Myocardial longitudinal strain	Speckle tracking echocardiography (STE)	38 39
Genetic polymorphisms	Polymorphism of the angiotensin converting enzyme	Blood cells	48-51
	Polymorphism of the serotonin	Blood cells	52-54
Demographics* (* Adjustment variables)	Age, gender, qualification, personal work status, ethnicity, life and occupational events	Questionnaire	10
Clinical measurements		Height, weight, blood pressure, heart rate, waist circumference	
Body composition	Muscle mass, fat mass, bone structure	Impedance meter	36
		Densitometry X-ray absorption	35
		Peripheral quantitative computed tomography (pQCT)	73-75
		Quantitative ultrasounds (QUS)	84
Psychology and quality of life	Depression	HAD (7 items)	57
	Anxiety	HAD (7 items) Hamilton scale for anxiety (7-item)	57
		State and Trait Anxiety Inventory (STAI)	58 59
	General health	General health questionnaire SF-36 (36 items)	71
	Quality of life	Brief Multidimensional Life Satisfaction Scale (11 items)	85
	Stress, fatigue, sleep	100 mm Visual analog scale	10
	Burnout	Maslach Burnout Inventory	56
	Mindfulness	Mindfulness Fribourg Mindfulness Inventory	22,23
	Coping	Brief COPE questionnaire (BCQ)	60
	Emotions	Emotion Regulation Questionnaire (ERQ)	61
	Perception of work	Karasek's Job Content Questionnaire	63
	Self-efficacy	Perceived self-efficacy scale	64
	Alexithymia	Toronto Alexithymia Scale (TAS-20)	65-67
	Illness Perception	Brief Illness Perception Questionnaire (B-IPQ)	68 69
	Metacognition	MetaCognition Questionnaire (MCQ-30)	70
Personal resources	Trait perception of workplace stress	Inner Correspondence/Peaceful Harmony with practices (ICPH) (17 items)	86
Lifestyle	Smoking, alcohol, coffee, food intake, etc.	Questionnaires	10
	Physical activity	Recent Physical Activity Questionnaire (RPAQ)	72
Alloplastic load	HbA1c, HDLc and LDL cholesterol, TG	EDTA tube	35

	Cortisol	Dry tube, serum isolation and deep-freezing	46 47
	DHEAS	Dry tube, serum isolation and deep-freezing	46 47
	Leptin	Dry tube, serum isolation and deep-freezing	35
	Ghrelin	Dry tube, serum isolation and deep-freezing	7 41
	BDNF	Dry tube, serum isolation and deep-freezing	42-44
	Pro-inflammatory Cytokines: IL-1β, IL-6, IL-1, TNFα	Dry tube, serum isolation and deep-freezing	35
	NPY	Dry tube, serum isolation and deep-freezing	45
	Telomere length	Blood; analysis by southern blot or PCR	55

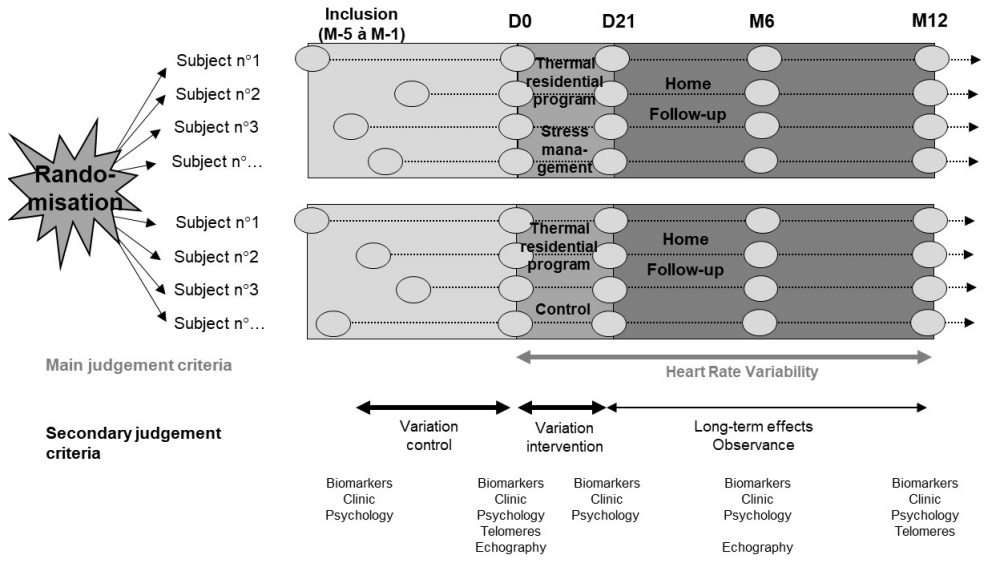
Figure Title: ObestiStress protocol

Figure Abbreviation: M months, D days

Supplementary files:

S1 - TIDieR checklist

S2 - patient consent form



Title: The ObesiStress protocol
M months, D day

105x59mm (300 x 300 DPI)

TIDieR
Template for Intervention Description and Replication

The TIDieR (Template for Intervention Description and Replication) Checklist*:

Information to include when describing an intervention and the location of the information

Item number	Item	Where located **	
		Primary paper (page or appendix number)	Other † (details)
	BRIEF NAME		
1.	Provide the name or a phrase that describes the intervention.	1	
	WHY		
2.	Describe any rationale, theory, or goal of the elements essential to the intervention.	5	
	WHAT		
3.	Materials: Describe any physical or informational materials used in the intervention, including those provided to participants or used in intervention delivery or in training of intervention providers. Provide information on where the materials can be accessed (e.g. online appendix, URL).	6-14	
4.	Procedures: Describe each of the procedures, activities, and/or processes used in the intervention, including any enabling or support activities.	6-14	
	WHO PROVIDED		
5.	For each category of intervention provider (e.g. psychologist, nursing assistant), describe their expertise, background and any specific training given.	N/A	
	HOW		
6.	Describe the modes of delivery (e.g. face-to-face or by some other mechanism, such as internet or telephone) of the intervention and whether it was provided individually or in a group.	8	
	WHERE		
7.	Describe the type(s) of location(s) where the intervention occurred, including any necessary infrastructure or relevant features.	8	

	WHEN and HOW MUCH		
8.	Describe the number of times the intervention was delivered and over what period of time including the number of sessions, their schedule, and their duration, intensity or dose.	___6/8_____	_____
	TAILORING		
9.	If the intervention was planned to be personalised, titrated or adapted, then describe what, why, when, and how.	__n/a_____	_____
	MODIFICATIONS		
10.‡	If the intervention was modified during the course of the study, describe the changes (what, why, when, and how).	___n/a_____	_____
	HOW WELL		
11.	Planned: If intervention adherence or fidelity was assessed, describe how and by whom, and if any strategies were used to maintain or improve fidelity, describe them.	___12_____	_____
12.‡	Actual: If intervention adherence or fidelity was assessed, describe the extent to which the intervention was delivered as planned.	_____	_____

** **Authors** - use N/A if an item is not applicable for the intervention being described. **Reviewers** – use '?' if information about the element is not reported/not sufficiently reported.

† If the information is not provided in the primary paper, give details of where this information is available. This may include locations such as a published protocol or other published papers (provide citation details) or a website (provide the URL).

‡ If completing the TIDieR checklist for a protocol, these items are not relevant to the protocol and cannot be described until the study is complete.

* We strongly recommend using this checklist in conjunction with the TIDieR guide (see *BMJ* 2014;348:g1687) which contains an explanation and elaboration for each item.

* The focus of TIDieR is on reporting details of the intervention elements (and where relevant, comparison elements) of a study. Other elements and methodological features of studies are covered by other reporting statements and checklists and have not been duplicated as part of the TIDieR checklist. When a **randomised trial** is being reported, the TIDieR checklist should be used in conjunction with the CONSORT statement (see www.consort-statement.org) as an extension of **Item 5 of the CONSORT 2010 Statement.** When a **clinical trial protocol** is being reported, the TIDieR checklist should be used in conjunction with the SPIRIT statement as an extension of **Item 11 of the SPIRIT 2013 Statement** (see www.spirit-statement.org). For alternate study designs, TIDieR can be used in conjunction with the appropriate checklist for that study design (see www.equator-network.org).

Formulaire de consentement

Titre de l'étude : **Obesi-Stress**
Coordinateur et Investigateur principal : Dr Frédéric Dutheil
Promoteur : **CHU Clermont-Ferrand**, 58 Rue de Montalembert, 63000 Clermont-Ferrand, France

Je soussigné(e)

M^{me}, M. *(rayer les mentions inutiles)* (*nom, prénom*) ……

Né(e) le ……

Demeurant ……

Déclare :

- que le Docteur (nom, prénom, tel) …… m'a proposé de participer à l'étude sus nommée,
- qu'il m'a expliqué en détail le protocole,
- qu'il m'a notamment fait connaître :
  - l'objectif, la méthode et la durée de l'étude
  - les contraintes et les risques potentiels encourus
  - mon droit de refuser de participer et en cas de désaccord de retirer mon consentement à tout moment
  - mon obligation d'inscription à un régime de sécurité sociale
  - que, si je le souhaite, à son terme, je serais informé(e) par le médecin investigateur de ses résultats globaux
  - que le Comité de Protection des Personnes Sud-Est VI a émis un avis favorable en date du 25/01/2018
  - que l'ANSM a délivré une autorisation pour cette étude
  - que dans le cadre de cette étude le promoteur, le CHU de Clermont-Ferrand a souscrit à une assurance couvrant cette recherche
- que j'ai répondu en toute bonne foi aux questions concernant mon état de santé.

Les informations relatives à l'étude recueillies par l'investigateur sont traitées confidentiellement. J'accepte que les données enregistrées à l'occasion de cette recherche puissent faire l'objet d'un traitement informatisé anonyme. J'ai bien noté que le droit d'accès prévu par la loi du 6 août 2004 relative à l'informatique, aux fichiers et aux libertés s'exerce à tout moment auprès du médecin qui me suit dans le cadre de la recherche et qui connaît mon identité. Je pourrai exercer mon droit de rectification et d'opposition auprès de ce même médecin, qui contactera le promoteur de la recherche.

Après avoir discuté librement et obtenu réponse à toutes mes questions, j'accepte librement et volontairement de participer à cette recherche biomédicale dans les conditions précisées dans le formulaire d'information et de consentement.

Nom et prénom du participant :	**Nom de l'investigateur :**
……	**Dr**
Date : . . / . . / 201.	**Date : . . / . . / 201.**
Signature, précédée de la mention « Lu et compris »:	**Signature :**

Ce document est à réaliser en 2 exemplaires originaux, dont le premier doit être gardé 15 ans par l'investigateur, un autre remis à la personne donnant son consentement.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRIT reporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	#3	Date and version identifier	Page 2
Funding	#4	Sources and types of financial, material, and other support	Page 14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Pages 1-2;14
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	Page 2

sponsor contact information			
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Page 14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	
Objectives	#7	Specific objectives or hypotheses	Page 5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	Page 6
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Pages 7-8

Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Pages 8-11
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Pages 6, 8, Figure 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Pages 6-7
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 7
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 6
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Pages 8-11
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Pages 11-12
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Pages 11-12
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple	Pages 11-12

		imputation)	
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	Page 24
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 13
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	
Declaration of	#28	Financial and other competing interests for principal	Page 14

interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 13
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	