BMJ Open

# Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes: an exercise intervention program for reducing the risk of fractures.
# Study protocol for a randomized controlled trial



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2018-027429 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 22-Oct-2018 |
| Complete List of Authors: | Balducci, Stefano; La Sapienza University, Department of Clinical and Molecular Medicine<br>Conti, Francesco; La Sapienza University, Rome, Department of Clinical and Molecular Medicine<br>Sacchetti, Massimo; Foro Italico University, Department of Human Movement and Sport Sciences<br>Russo, Cosimo; Metagym<br>Argento, Giuseppe; Sant'Andrea University Hospital, Radiology Unit<br>Haxhi, Jonida; La Sapienza University, Department of Clinical and Molecular Medicine<br>Orlando, Giorgio; Foro Italico University, Department of Human Movement and Sport Sciences<br>Rapisarda, Gianvito; La Sapienza University, Department of Clinical and Molecular Medicine<br>D'Errico, Valeria; La Sapienza University, Department of Clinical and Molecular Medicine<br>Cardelli, Patrizia; La Sapienza University, Department of Clinical and Molecular Medicine<br>Pugliese, Luca; Sant'Andrea University Hospital, Radiology Unit<br>Laghi, Andrea; Sant'Andrea University Hospital, Radiology Unit<br>Vitale, Martina; La Sapienza University, Department of Clinical and Molecular Medicine<br>Bollanti, Lucilla; La Sapienza University, Department of Clinical and Molecular Medicine<br>Zanuso , S; Coventry University, Centre for Applied Biological & Exercise Sciences<br>Nicolucci, Antonio; Center for Outcomes Research and clinical Epidemiology (CORE), Pescara, Italy.,<br>Pugliese, Giuseppe; La Sapienza University, Rome, Department of Clinical and Molecular Medicine |
| Keywords: | General diabetes < DIABETES & ENDOCRINOLOGY, Diabetes & endocrinology < INTERNAL MEDICINE, Bone diseases < ORTHOPAEDIC & TRAUMA SURGERY, PREVENTIVE MEDICINE |
| | |

# Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes: an exercise intervention program for reducing the risk of fractures

## Study protocol for a randomized controlled trial

Stefano Balducci [1,2,3], Francesco G. Conti [1,2], Massimo Sacchetti [4], Cosimo R. Russo [5], Giuseppe Argento [6], Jonida Haxhi [2,3,4], Giorgio Orlando [4*], Gianvito Rapisarda [2,3], Valeria D'Errico [2,3], Patrizia Cardelli [1,7], Luca Pugliese [6†], Andrea Laghi [6,8], Martina Vitale [1,2], Lucilla Bollanti [1,2], Silvano Zanuso [9], Antonio Nicolucci [10], and Giuseppe Pugliese [1,2]; for the SWEET BONE Investigators [‡].

[1] Department of Clinical and Molecular Medicine, ''La Sapienza'' University, Rome, Italy; [2] Diabetes Unit, Sant'Andrea University Hospital, Rome, Italy; [3] Metabolic Fitness Association, Monterotondo, Rome, Italy; [4] Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy; [5] Metagym Fitness Centre, Florence, Italy; [6] Radiology Unit, Sant'Andrea University Hospital, Rome, Italy; [7] Laboratory of Clinical Chemistry, Sant'Andrea University Hospital, Rome, Italy; [8] Department of Radiological Sciences, Oncology and Pathology, ''La Sapienza'' University, Rome, Italy; [9] Centre for Applied Biological & Exercise Sciences, Faculty of Health & Life Sciences, Coventry University, Coventry, UK; and [10] Centre for Outcomes Research and Clinical Epidemiology (CORESEARCH), Pescara, Italy.

Current address: [*] School of Healthcare Science, Faculty of Science & Engineering, Manchester Metropolitan University, Manchester, UK; [†] Department of Diagnostic Imaging, "Tor Vergata" University, Rome, Italy.

[‡] See Appendix A for the complete list of the SWEET_BONE Investigators.

**Corresponding Author:** Giuseppe PUGLIESE, MD, PhD, Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, Via di Grottarossa, 1035-1039 - 00189 Rome, Italy; Phone: +39-0633775440; Fax: +39-0633776327; E-mail: giuseppe.pugliese@uniroma1.it.

**Key words:** type 2 diabetes; bone quality; bone mass; bone fractures; exercise; physical fitness.

**Word count:** abstract 299; text 4,000.

## Abstract

**Introduction.** Type 2 diabetes (T2D) is associated with an increased fracture risk despite normal-to-increased bone mineral density, suggesting reduced bone quality. Exercise may be effective in reducing fracture risk in T2D individuals by ameliorating muscle dysfunction and reducing risk of fall, though it is unclear whether it can improve bone quality.

**Methods and analysis.** The "Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in T2D" is an open-label, assessor-blinded, randomized controlled trial comparing an exercise training program of 2-year duration, specifically designed for improving bone quality and strength, with standard care in T2D individuals. Two-hundred T2D patients aged 65-75 years will be randomized 1:1 to supervised exercise training or standard care, stratified by gender, age ≤ or >70 years, and non-insulin or insulin treatment. The intervention consists of two weekly supervised exercise sessions, each starting with 5 min of warm-up, followed by 20 min of aerobic training using weighted vests, 30 min of strength and power training, and 20 min of core stability, balance, and flexibility training. The primary endpoint is baseline to end-of-study change in trabecular bone score, a parameter of bone quality consistently shown to be reduced in T2D. Secondary endpoints include changes in other potential measures of bone quality, as assessed by quantitative ultrasound and peripheral quantitative computer tomography; bone mass; markers of bone turnover; muscle strength, mass, and power; balance and gait. Falls and asymptomatic and symptomatic fractures will be evaluated over 7 years, including a 5-year post-trial follow-up. The superiority of the intervention will be assessed by comparing between-groups baseline to end-of-study changes.

**Ethics and dissemination.** This study was approved by the institutional ethics committee. Written informed consent will be obtained from all participants. The study results will be submitted for peer-reviewed publication.

**Registration details.** ClinicalTrials.gov, NCT02421393.

## Strengths and limitations of this study

- This is the first study investigating whether a specifically designed exercise training program of 2-year duration is effective in improving bone quality and strength in patients with type 2 diabetes, thus reducing the increased fracture risk characterizing these individuals.
- A wide range of parameters of bone quality and strength is assessed, together with measures of bone mass and muscle mass, strength and power, which all may affect fracture risk.
- All the physicians, exercise specialists, and outcome assessors have been specifically trained for conducting in this trial and participated in a pilot study aimed at setting up the trial protocol.
- The efficacy of the intervention in reducing falls and fractures will be assessed over an extended 7-year period, including a 5-year post-intervention follow-up.
- Generalizability and implementation in clinical practice of this approach will require further investigation and validation in different cohorts or contexts.

## Introduction

Risk of fracture is significantly increased in type 1 diabetes (T1D) and, to a lower extent, in type 2 diabetes (T2D).[1, 2] However, bone mineral density (BMD), the strongest predictor of fractures in individuals with osteoporosis,[3] was found almost consistently reduced in T1D patients, whereas it was reported to be normal or even increased in T2D individuals, despite the increased fracture risk.[1] Notably, in T2D patients, the increase in fracture risk remained after adjustment for BMD [4-6] and also for falls,[4, 5, 7] which are more frequent in older individuals with T2D than in those without.[8] In addition, as compared with non-diabetic individuals, patients with T2D had a higher T-score for a similar fracture risk.[9] While the preserved bone mass may account for the lower fracture risk in T2D versus T1D, a reduced bone quality has been claimed to explain the discrepancy between normal BMD and increased fracture risk in T2D patients.[1, 2]

Bone quality is determined by (a) bone architecture, including geometry (macro-architecture) and micro-architecture; and (b) material properties, including mineralization and collagen cross-links, which in turn are influenced by bone turnover as well as by accumulation of microdamage and microstructural discontinuities such as microporosity and lamellar boundaries.[10, 11] While conventional dual-energy X-ray absorptiometry (DXA) measures bone mass, several techniques have been proposed for non-invasive assessment of bone quality.[12, 13] The trabecular bone score (TBS) is a gray-level texture measurement based on the use of experimental variograms of 2D projection images acquired during a DXA lumbar spine scan.[14] It was consistently found to be reduced in T2D patients with versus those without fracture [15] and in T2D versus non-diabetic individual, [16-21], and to predict fractures in both diabetic and non-diabetic subjects, independent of BMD.[16] Quantitative ultrasound (QUS), usually performed at the heel, provides an estimate of BMD,[22] which predicted fracture risk better than DXA-derived BMD in older women with T2D.[23] In addition, QUS evaluates parameters of bone quality, including micro-architecture and material properties.[24,25] However, QUS-derived bone structure measures were not consistently lower in T2D patients with versus those without fracture [26, 27] and in T2D versus non-diabetic individuals.[28, 29] In

addition to volumetric BMD (vBMD), low- and high-resolution peripheral quantitative computed tomography (pQCT) provides measures of bone geometry and architecture, which are surrogates of bone quality and strength.[12]. Higher cortical porosity and lower calculated strength were also reported in T2D patients with versus those without fracture [30, 31] and in T2D versus non-diabetic individuals.[32-35]

Physical activity (PA)/exercise has been suggested as an effective tool for improving bone health in subjects at high fracture risk by applying mechanical strain to the skeleton. It is known that both compressive loading from weight bearing and muscle contraction deform the osteocytes, which function as strain transducers by signalling osteoblasts, osteoclasts, and other cells to produce or break down bone, thus regulating bone mass and quality.[36, 37] In the absence of muscle activity against gravity, the biochemical signals result in increased resorption.[38] Conversely, appropriate types, amounts, and directions of strain result in bone mass maintenance, bone formation, and/or changes in bone geometry that improve bone strength.[39]

Exercise was shown to improve BMD to a relatively small, but clinically significant extent.[40] There is also a great deal of evidence from observational studies that higher PA levels are associated with fewer fractures in community-dwelling populations,[41] and postmenopausal women who performed spinal extension exercises showed a lower incidence of vertebral fractures.[42] Combination of diet and exercise was shown to provide greater improvement in physical function than either intervention alone.[43] Moreover, exercise training prevented the increase in bone turnover and attenuated the decrease in hip BMD associated with diet-induced weight loss,[44] and resistance exercise alone or combined aerobic and resistance exercise attenuated diet-induced decrease in muscle mass and BMD more than aerobic training alone.[45] Resistance exercise was also shown to decrease falls and risk of falls, especially when focused on strengthening the hip and ankle muscle groups which are involved in balance maintenance.[46]

These observations indicate that PA/exercise, especially of resistant type, may be effective in reducing fracture risk in T2D patients by ameliorating the decreased muscle mass, strength, and quality [47] and reducing the increased falls and risk of fall characterizing these individuals.[8] However, as BMD is usually preserved in T2D, it is unclear whether PA/exercise may reduce fracture risk also by directly improving bone

health in individuals suffering from this condition. Indeed, to date, there are no data on whether exercise training is effective in ameliorating bone quality and whether improved quality results in increased bone strength and reduced risk of fractures in subjects with T2D.

The "Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in T2D" is aimed at investigating the efficacy of a specific exercise intervention program of two-year duration on parameters of bone quality and strength in patients with T2D.

## Methods and analysis

### Trial design

The SWEET BONE is an open-label, assessor-blinded, parallel, superiority randomized controlled trial (RCT) comparing a specifically designed exercise intervention program with standard care in individuals with T2D. The trial flow chart is shown in Figure 1.

### Participants

This study will enrol patients with T2D (defined by the American Diabetes Association criteria [48]) of at least 5-year duration, of both sexes, aged 65-75 years. Additional requirements will be: physically inactivity (i.e. insufficient amounts of PA according to current guidelines) [49] and sedentary lifestyle (i.e. more than 8 hours/day spent in a sitting or reclining posture) [50] from ≥6 months; body mass index (BMI) 27-40 kg/m$^2$; ability to walk 1.6 Km without assistance; a Short Battery Performance Test score ≥4; and eligibility after cardiologic evaluation. All patients attending the Diabetes Clinic will be evaluated for eligibility.

In order to preserve high internal validity and reduce risk of adverse events, the criteria listed in Table 1 will be used to exclude individuals with conditions limiting or contraindicating PA, affect conduct of the trial, reduce lifespan, and/or affect the safety of intervention. Among exclusion criteria there are treatment with anti-fracture agents, oestrogens, aromatase inhibitors, testosterone, corticosteroids and/or glitazones; previous documented non-traumatic fractures; spinal deformity index (SDI) >3 (and >2 in a single vertebra); and a T score <-2.5 at spine/hip at DXA. Subjects with haemoglobin (Hb) $A_{1c}$ >9.0%, blood pressure (BP) >150/90 mmHg, and/or vitamin D <10 ng/ml will be re-evaluated for eligibility after receiving appropriate glucose- and BP-lowering therapy and a 6-week treatment with cholecalciferol 25.000 IU/week.

A sample of 50 non-diabetic subjects meeting the inclusion/exclusion criteria reported above (except for T2D-related criteria) and matched 1:4 by age, gender, and BMI will serve as controls for baseline measures.

**Investigators**

All the SWEET-BONE physicians, exercise specialists, and outcome assessors (see Appendix A) have been specifically trained for conducting this RCT and participated in a pilot study aimed at setting up the trial protocol.

To minimize dropout and reduce the attrition bias due to missing data, both physicians and exercise specialists have been instructed on how to promote participant retention in the trial. In particular, they have been recommended to contact participants at regular intervals, to keep up to date contact information for participants, and to collect complete data for both the primary and secondary outcomes, regardless of whether subjects continue to receive the assigned intervention.

**Recruitment**

Starting on 1 November, 2018, 200 patients will be recruited at the Diabetes Unit of Sant'Andrea University Hospital, a tertiary referral, outpatients Diabetes Clinic in Rome, Italy. All patients attending the clinic will be evaluated for eligibility. It has been calculated that at least 500 of patients seen each year meet the inclusion/exclusion criteria for this trial. The recruitment process will include four visits designated as R1, R2, R3, and R4.

On R1, eligible patients will be identified based on medical history, clinical examination, and results of the Minnesota leisure-time PA questionnaire. Then, patients will be asked to sign an informed consent and will be registered in the SWEET-BONE database available at http://www.metabolicfitness.it/. Finally, patients will undergo a cardiologic examination, including a resting electrocardiogram (ECG) and, based on clinical judgment, an echocardiogram and/or an ECG treadmill test.

On R2, baseline anthropometrical and clinical parameters and blood and urine samples for biochemical testing will be taken. Subsequently, participants will perform a Short Battery Performance Test and undergo measurement of ankle-brachial index and fundus evaluation. Finally, patients will attend a run-in session for familiarization with testing devices and protocols for the assessment of physical fitness.

On R3, patients will be asked to fill in the History of Falls questionnaire, the Physical Activity Scale for the Elderly (PASE) questionnaire, and a questionnaire for musculoskeletal (MS) symptoms. Then, participants will undergo x-ray of dorso-lumbar spine for vertebral morphometry, and total body and segmental DXA. Finally, they will attend another run-in session for familiarization prior to the assessment of physical fitness.

On R4, patients will receive a standard treatment regimen including nutritional therapy and prescription of pharmacological agents, as needed. Then, they will undergo the following procedures: peripheral QCT (pQCT), calcaneal QUS, and dynamometry. Finally, patients will be subjected to the assessment of physical fitness and will be informed about group assignment.

**Randomization**

Patients will be randomized 1:1 to supervised exercise training on top of standard care (exercise, EXE, group; n=100) versus standard care (control, CON, group; n=100) for 24 months.

Randomization will be stratified by gender (males versus females), age (65-70 versus 71-75) and type of diabetes treatment (non-insulin versus insulin), using a permuted-block randomization software which randomly varies the block size. To ensure allocation concealment, randomization will be centralized at the Centre for Outcomes Research and Clinical Epidemiology (CORESEARCH), and the group assignment of each newly recruited patient will be communicated to the investigators by telephone call.

After randomization, participants and care providers (physicians and exercise specialists) will not be blinded to group assignment, as blinding in unfeasible in exercise intervention studies.

**Follow-up**

Participants from both groups will attend four follow-up visits, designated as F1, F2, F3, and F4, after 6, 12, 18, and 24 months, respectively.

On F1, F2, and F3, patients will undergo a routine diabetes visit, with eventual adjustment of dietary and pharmacological prescriptions, and will be asked to fill in the History of Falls, PASE, and MS questionnaire. On

F2 only, intermediate anthropometrical and clinical parameters and blood and urine samples for centralized biochemical testing will be taken.

On F4, end-of study anthropometrical and clinical parameters and blood and urine samples for biochemical testing will be taken. Then, participants will be asked to fill in the History of Falls, PASE, and MS questionnaires and to perform a Short Battery Performance Test. On different days, patients will undergo x-ray of dorso-lumbar spine for vertebral morphometry, total body and segmental DXA, pQCT, calcaneal QUS, and dynamometry and assessment of physical fitness.

**Post-trial follow-up**

Participants will be followed every 6 months for additional 5 years for routine diabetes visits. On this occasion, they will be asked to provide clinical records on eventual fractures, to fill in the History of Falls, PASE, and MS questionnaires, and to perform a Short Battery Performance Test. At the end of the 5-year post-trial follow-up, participants will undergo vertebral morphometry to detect asymptomatic fractures.

**Intervention**

The training program for the EXE group will consist of two 75-min weekly sessions, supervised by an exercise specialist in the gym facility of the Metabolic Fitness Association (Figure 2). We conducted a pilot study on a small sample of T2D patients meeting the inclusion/exclusion criteria for this RCT in order to set up the training program and preliminarily evaluate both the efficacy and safety of the intervention.

Each session will start with 5 min of warm up, followed by 20 min of aerobic, and 30 min of strength and power training consisting of 15 min of resistance exercises and 15 min of “weight bearing” exercises.

The session will end with 20 min of core stability training (8 min), which improves the ability to control the position and movement of the central portion of the body and targets the deep abdominal muscles that assist in posture maintenance and arm and leg movements, balance training (8 min), and flexibility training (4 min).

Starting at month 2, a weighted vest will be worn during aerobic training, weight bearing exercises, and any occupational, home, and leisure-time PA. Patients will be asked to record in a daily diary the time spent

wearing the weighted vest outside the sessions. Weight of vests will be 2% of body weight and will increase thereafter by 2% every 6 months (up to 8%).

**Standard care**

All patients will be subjected to a treatment regimen aimed at achieving glycaemic, lipid, BP and body weight targets, as established by current guidelines and including nutritional therapy and glucose-, lipid- and BP-lowering agents as needed.[48] Vitamin D will be supplemented to maintain levels higher than 30 ng/ml.

At intermediates routine diabetes visits, drugs will be adjusted to attain target levels, following a pre-specified algorithm, and changes will be recorded into the SWEET-BONE database.

**Outcomes**

The primary endpoint is baseline to end-of-study change in TBS, based on previous reports showing that it is consistently lower in T2D versus control subjects [16-21].

Secondary endpoints include (a) other potential measures of bone quality, as assessed by QUS and pQCT; (b) bone mass (BMD); (c) markers of bone turnover; (d) body composition; (e) muscle strength, mass, and power; (f) balance and gait; (g) number of falls; and (h) asymptomatic and symptomatic fractures. Falls and fractures will be evaluated over 7 years (i.e., including the 5-year post-trial follow-up).

PA level, other components of physical fitness, i.e., cardio-respiratory fitness and flexibility, MS symptoms, modifiable cardiovascular risk factors, medications, and global coronary heart disease (CHD) and stroke 10-year risk scores will be also evaluated.

The assessors of outcome measures will be blinded to group assignment.

**Measurements**

***Bone mass and quality.*** Bone mass will be assessed by DXA scans of the posterior-anterior lumbar spine (L1 to L4) and total femur using Hologic QDR 4500 W 2000 (Hologic, Bedford, MA, USA). Areal BMD (aBMD, g/cm$^2$) in the lumbar spine and femoral neck will be recorded and the corresponding T scores and Z scores will be obtained. Composite indices of femoral neck strength will be also computed from the femoral neck axis length

(FNAL) and width (FNW), femoral neck areal BMD, and body size, i.e. compression strength index (CSI), bending strength index (BSI), and impact strength index (CSI).[51] TBS will be then measured using the Hologic TBS Insight software (Hologic). QUS measurements will be performed at the heel using the Sahara® Clinical Bone Sonometer (Technologic, Turin, Italy). Broadband ultrasound attenuation (BUA; dB/MHz) and speed of sound (SOS; m/s) will be measured, and the quantitative ultrasound index (QUI) will be then calculated. BMD will be also estimated from QUS measurements (eBMD, g/cm$^2$). Bone density and macroarchitecture will be evaluated by pQCT using an XCT-2000 pQCT scanner (Norland Stratec, Stratec, Pforzheim, Germany).[52] Slices (2.5 mm) will be obtained at the 4%, 14%, 38%, and 66% sites of the left tibia and at the 4% site of the nondominant radius. At the metaphyses (4% site) of tibia and radius, total vBMD (Tot-vBMD) and Trab-vBMD (mg/cm$^3$) will be measured. At the 14% site of the tibia, cortical bone area (Cort-A, mm$^2$) and cortical bone mineral content (Cort-BMC, mg/cm), two markers of resistance to compressive and tensile loads, will be measured, and the section modulus will be calculated from the antero-posterior, latero-lateral, and polar moments of inertia (Ix, Iy, and Ip, respectively) and used to obtain the stress-strain index (SSI, mm$^3$), a surrogate measure of resistance to bending (xSSI and ySSI) and torsional (pSSI) loads. At the 38% site of the tibia, cortical vBMD (Cort- vBMD, mg/cm$^3$) and total cross-sectional area (Tot-CSA, mm$^2$) will measured, together with calculation of cortical thickness (CT, mm) and circularity index (CI), a proxy of tibial geometrical load adaptation.

***Markers of bone turnover.*** Serum calcium and phosphorus, 25OH Vitamin D, and parathyroid hormone will be measured, together with the following markers of bone turnover: total and bone-specific alkaline phosphatase, osteocalcin, and procollagen I intact N-terminal, for bone formation, and C-terminal telopeptide of type I collagen, tartrate-resistant acid phosphatase 5b, sclerostin, and Dickkopf-1, for bone resorption. These measurements will be centralized at the Laboratory of Clinical Chemistry of Sant'Andrea University Hospital, an accredited and ISO9001 certified structure, using the methods reported in Table 2.

***Body composition.*** Total body DXA will, be used to evaluate body composition, with measurement of total body lean mass and total body fat mass.

***Muscle strength.*** Isometric muscle strength will be also assessed by means of a strain gauge tensiometer (Digimax, Mechatronic GmbH, Germany), as previously reported.[53] Maximal voluntary contractions are performed at the shoulder press (Technogym, Gambettola, Italy) along the sagittal plan, with a 45° and 90° angle at the elbow and between the upper arm and the trunk, respectively, for the upper body, and at the leg extension machine (Technogym), with a 90° angle at the knee and the hip, for the lower body. Values will be expressed in Nm for two arms.

***Muscle cross-sectional area.*** The cross-sectional areas of muscles of the leg will be measured by pQCT at the 66% site of the tibia at the end of bone assessments.[54]

***Physical fitness.*** Physical fitness will be evaluated at baseline, end-of-study and, in the EXE group, also at month 6, 12, and 18, in order to adjust training loads. Cardio-respiratory fitness, muscle fitness, and flexibility will be assessed by a sub-maximal evaluation of oxygen consumption at 80% of the maximal heart rate to predict maximal oxygen consumption ($VO_{2max}$), a maximal repetition (or 5-8 RM) to predict one-repetition maximum (1-RM), and a standard bending test, respectively, as previously reported.[55, 56]

***Balance, gait and power.*** A "Short Battery Performance Test" will be performed for the assessment of balance (side-by-side stand, semi-tandem stand and tandem stand), gait (gait speed test) and power (chair stand test).[57]

***Number of falls.*** Falls will be recorded using the 17-item History of Falls questionnaire (see Appendix B1).[58]

***Symptomatic and asymptomatic fractures.*** Patients will be interviewed to record symptomatic fractures, which will be adjudicated based on clinical and radiographic records. Asymptomatic fractures will be identified by vertebral morphometry.

***PA level.*** The level of PA will be evaluated throughout the study by asking patients to fill in the PASE questionnaire (see Appendix B2), a validated instrument for the measurement of PA level in individuals aged ≥65 years.[59] The amount of supervised exercise in the EXE group, will be measured as previously reported.[55, 56]

***MS symptoms.*** MS symptoms will be evaluated by a 50-item self-report questionnaire (see Appendix B3), which investigates shoulder, arm, elbow, wrist, hand, spine, hip, knee, ankle, and foot problems.[60]

***Cardiovascular risk factors and scores.*** The BMI will be calculated from body weight and height, while waist circumference will be taken at the umbilicus and BP will be recorded with a sphygmomanometer after a five-minute rest with the patient seated with the arm at the heart level. Moreover, blood and urine samples will be taken for measuring the biochemical parameters reported in Table 3 at the Laboratory of Clinical Chemistry of Sant'Andrea University Hospital. Global and fatal CHD and stroke 10-year risk scores will be calculated using the United Kingdom Prospective Diabetes Study (UKPDS) risk engine.[61] Cardiovascular risk factors and scores will be assessed at baseline, year 1, and end-of-study, though measurements will be eventually performed also at month 6 and 18, in order to adjust treatment.

**Adverse events**

Adverse events will be reported at intermediate visits and, for EXE subjects, also at supervised sessions, by completing a standard form.

The risk of injuries and other adverse events during the training sessions will be covered by an insurance (N. 390-01583709-14010, HDI-Gerling Industrie Versicherung AG, Leipzig, Deutschland).

**Data collection, storage and security**

Data collected via web into the SWEET-BONE database will be saved to a password-protected server in the Metabolic Fitness Association. These data will be accessed only by members of the research team.

Once all data have been uploaded to the server, they will be securely deleted from the recording devices. Patient questionnaire data will be made anonymous and stored in locked filing cabinets.

**Statistical analysis**

Sample size calculation was based on our pilot study showing that TBS was 1.225±0.085 in T2D individuals versus 1.255±0.067 in non-diabetic controls. To observe an improvement in the EXE group which allows to

bridge the gap of 0.030 with non-diabetic controls with a statistical power of 90% (α=0.05) by unpaired t test, 89 patients per arm are needed (178 total). A sample of 200 patients allows to tolerate an 11% dropout rate.

The $\chi^2$ test or, where appropriate, the Fisher's exact test, for categorical variables, and the Student's t test or the corresponding nonparametric Mann-Whitney test for continuous variables will be utilized to compare patients' characteristics at baseline. The intention-to-treat analysis will be applied to all randomized patients with a baseline and an end-of-study value. The superiority of the intervention on the primary and secondary endpoints will be assessed using the unpaired t-test or the Mann Whitney U test, by comparing between-groups changes from baseline to end-of-study. Within-group end-of-study versus baseline values will be compared using the paired t-test or the Wilcoxon signed ranks test.

To account for change in medication throughout the study period, which might affect bone parameters, we will perform both multiple regression and sensitivity analyses. In the regression models, the dependent variable will be represented by baseline to end-of-study changes. Treatment at baseline and treatment initiation during the study will be included in the model as dichotomous variables (yes versus no), whereas drug dosage will be not taken into consideration. Sensitivity analysis will be conducted by comparing study arms after exclusion of patients who modified treatment.

In order to treat attrition, we will assume that data are missing at random and repeated measures models with an autoregressive correlation type matrix will be applied to account for both missingness at random and potential correlation within subjects.[62] Finally, to guarantee replicability and avoid outcome selective reporting, a fully specified statistical analysis plan will be written before unmasking.

The effect of the following subgroups will be explored separately: gender (males versus females), age (65-70 versus 71-75) and type of diabetes treatment (non-insulin versus insulin).

Statistical analyses will be performed by at the CORESEARCH using SAS software release 9.3 (Cary, NC, USA).

## Ethics and Dissemination

The research protocol (version #3, February 28, 2013), which follows the SPIRIT guideline (see Appendix C), complies with the Declaration of Helsinki. It has been approved by the Ethics Committee of Sant'Andrea University Hospital on 21 March, 2013 (Prot. n. 2583/2013) and has been registered with ClinicalTrials.gov on 20 April. 2014 (NCT02421393; URL https://clinicaltrials.gov/ct2/show/NCT02421393) (see Appendix D). Important protocol amendments (e.g., changes to eligibility criteria, outcomes, analyses) will be communicated to relevant parties, i.e., investigators, trial participants, trial registry (ClinicalTrials.gov), and the Ethics Committee of Sant'Andrea University Hospital.

All participants will provide written informed consent (see Appendix E) following verbal and written explanation of the study protocol and the opportunity to ask questions. Participants will not be provided with an honorarium and will be free to withdraw from the trial at any time without prejudice to future treatment.

To the best of our knowledge, the SWEET-BONE is the first study investigating whether a specifically designed exercise training program is effective in improving bone quality and strength in patients with T2D, thus potentially reducing the increased fracture risk characterizing these individuals despite preserved bone mass. The beneficial effects on bone quality would be additional to those on muscle strength and mass and risk of fall, which may reduce per se the risk of fracture. However, generalizability and implementation in clinical practice of this approach will require further investigation and validation in different cohorts or contexts.

Results will be presented at scientific meetings and published in peer-reviewed journals. All publications and presentations related to the study will be authorized and reviewed by the study investigators. The ICMJE Recommendations will be adopted for authorship [63].

After publication of results, public access to the full protocol, participant-level dataset, and statistical code will be eventually granted upon request.

## References


1. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. *Osteoporos Int.* 2007;18:427–444.
2. Janghorbani M, Van Dam RM, Willett WC, et al. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *Am J Epidemiol.* 2007;166:495–505.
3. Hernlund E, Svedbom A, Ivergård M, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Arch Osteoporos.* 2013;8:136.
4. Schwartz AV, Sellmeyer DE, Ensrud KE, et al. Older women with diabetes have an increased risk of fracture: a prospective study. *J Clin Endocrinol Metab.* 2001;86:32–38.
5. Strotmeyer ES, Cauley JA, Schwartz AV, et al. Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: The Health, Aging, and Body Composition Study. *Arch Intern Med.* 2005;165:1612–1617.
6. Napoli N, Strotmeyer ES, Ensrud KE, et al. Fracture risk in diabetic elderly men: the MrOS study. *Diabetologia.* 2014;57:2057–2065.
7. Bonds DE, Larson JC, Schwartz AV, et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. *J Clin Endocrinol Metab.* 2006;91:3404–3410.
8. Schwartz AV, Hillier TA, Sellmeyer DE, et al. Older women with diabetes have a higher risk of falls: a prospective study. *Diabetes Care.* 2002;25:1749–1754.
9. Schwartz AV, Vittinghoff E, Bauer DC, et al. Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. *JAMA.* 2011;305:2184–2192.
10. Fonseca H, Moreira-Gonçalves D, Coriolano HJ, et al. Bone quality: the determinants of bone strength and fragility. *Sports Med.* 2014;44:37–53.

11. Farr JN, Khosla S. Determinants of bone strength and quality in diabetes mellitus in humans. *Bone.* 2016;82:28–34.

12. Rubin MR, Patsch JM. Assessment of bone turnover and bone quality in type 2 diabetic bone disease: current concepts and future directions. *Bone Res.* 2016;4:16001.

13. Jiang N, Xia W. Assessment of bone quality in patients with diabetes mellitus. *Osteoporos Int.* 2018 May 7.

14. Bousson V, Bergot C, Sutter B, et al. Trabecular bone score (TBS): available knowledge, clinical relevance, and future prospects. *Osteoporos Int.* 2012;23:1489–1501.

15. Zhukouskaya VV, Eller-Vainicher C, Gaudio A, et al. The utility of lumbar spine trabecular bone score and femoral neck bone mineral density for identifying asymptomatic vertebral fractures in well-compensated type 2 diabetic patients. *Osteoporos Int.* 2016;27:49–56.

16. Leslie WD, Aubry-Rozier B, Lamy O, et al. TBS (trabecular bone score) and diabetes-related fracture risk. *J Clin Endocrinol Metab.* 2013;98:602–609.

17. Dhaliwal R, Cibula D, Ghosh C, et al. Bone quality assessment in type 2 diabetes mellitus. *Osteoporos Int.* 2014;25:1969–1973.

18. Kim JH, Choi HJ, Ku EJ, et al. Trabecular bone score as an indicator for skeletal deterioration in diabetes. *J Clin Endocrinol Metab.* 2015;100:475–482.

19. Choi YJ, Ock SY, Chung YS. Trabecular Bone Score (TBS) and TBS-adjusted fracture risk assessment tool are potential supplementary tools for the discrimination of morphometric vertebral fractures in postmenopausal women with type 2 diabetes. *J Clin Densitom*. 2016;19:507–514.

20. Holloway KL, De Abreu LLF, Hans D, et al. Trabecular bone score in men and women with impaired fasting glucose and diabetes. *Calcif Tissue Int.* 2018;102:32–40.

21. Rianon N, Ambrose CG, Buni M, et al. Trabecular bone score is a valuable addition to bone mineral density for bone quality assessment in older Mexican American women with type 2 diabetes. *J Clin Densitom.* 2018;21:355–359.

22. Glüer CC. Quantitative ultrasound techniques for the assessment of osteoporosis: expert agreement on current status. The International Quantitative Ultrasound Consensus Group. *J Bone Miner Res.* 1997;12:1280–1288.

23. Patel S, Hyer S, Tweed K, et al. Risk factors for fractures and falls in older women with type 2 diabetes mellitus. *Calcif Tissue Int.* 2008;82:87–91.

24. Padilla F, Jenson F, Bousson V, et al. Relationships of trabecular bone structure with quantitative ultrasound parameters: In vitro study on human proximal femur using transmission and backscatter measurements. *Bone.* 2008;42:1193–1202.

25. Bouxsein ML, Radloff SE. Quantitative ultrasound of the calcaneus reflects the mechanical properties of calcaneal trabecular bone. *J Bone Miner Res.* 1997;12:839–846.

26. Patel S, Hyer S, Tweed K, et al. Risk factors for fractures and falls in older women with type 2 diabetes mellitus. *Calcif Tissue Int.* 2008;82:87–91.

27. Yamaguchi T, Yamamoto M, Kanazawa I, et al. Quantitative ultrasound and vertebral fractures in patients with type 2 diabetes. *J Bone Miner Metab.* 2011;29:626–632.

28. Tao B, Liu JM, Zhao HY, et al. Differences between measurements of bone mineral densities by quantitative ultrasound and dual-energy X-ray absorptiometry in type 2 diabetic postmenopausal women. *J Clin Endocrinol Metab.* 2008;93:1670–1675.

29. Bulló M, Garcia-Aloy M, Basora J, et al. Bone quantitative ultrasound measurements in relation to the metabolic syndrome and type 2 diabetes mellitus in a cohort of elderly subjects at high risk of cardiovascular disease from the PREDIMED study. *J Nutr Health Aging*. 2011;15:939–944.

30. Patsch JM, Burghardt AJ, Yap SP, et al. Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. *J Bone Miner Res.* 2013;28:313–324.

31. Samelson EJ, Demissie S, Cupples LA, et al. Diabetes and deficits in cortical bone density, microarchitecture, and bone size: Framingham HR-pQCT Study. *J Bone Miner Res.* 2018;33:54–62.

32. Burghardt AJ, Issever AS, Schwartz AV, et al. High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab.* 2010;95:5045–5055.

33. Farr JN, Drake MT, Amin S, et al. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. *J Bone Miner Res.* 2014;29:787–795.

34. Paccou J, Ward KA, Jameson KA, et al. Bone microarchitecture in men and women with diabetes: the importance of cortical porosity. *Calcif Tissue Int.* 2016;98:465–473.

35. Nilsson A, Sundh D, Johansson L, et al. Type 2 diabetes mellitus is associated with better bone microarchitecture but lower bone material strength and poorer physical function in elderly women: a population-based study. *J Bone Miner Res.* 2017;32:1062–1071.

36. Arnett TR. Osteocytes: regulating the mineral reserves? *J Bone Miner Res.* 2013;28:2433–2435.

37. Kerschnitzki M, Kollmannsberger P, Burghammer M, et al. Architecture of the osteocyte network correlates with bone material quality. *J Bone Miner Res.* 2013;28:1837–1845.

38. Skerry TM, Suva LJ. Investigation of the regulation of bone mass by mechanical loading: from quantitative cytochemistry to gene array. *Cell Biochem Funct.* 2003;21:223–229.

39. Sinaki M, Pfeifer M, Preisinger E, et al. The role of exercise in the treatment of osteoporosis. *Curr Osteoporos Rep.* 2010;8:138–144.

40. Howe TE, Shea B, Dawson LJ, et al. Exercise for preventing and treating osteoporosis in postmenopausal women. *Cochrane Database Syst Rev.* 2011;(7):CD000333.

41. Karlsson M. Does exercise reduce the burden of fractures? A review. *Acta Orthop Scand.* 2002;73:691–705.

42. Sinaki M, Itoi E, Wahner HW, et al. Stronger back muscles reduce the incidence of vertebral fractures: a prospective 10 year follow-up of postmenopausal women. *Bone.* 2002;30:836–841.

43. Villareal DT, Chode S, Parimi N, et al. Weight loss, exercise, or both and physical function in obese older adults. *N Engl J Med.* 2011;364:1218–1229.

44. Shah K, Armamento-Villareal R, Parimi N, et al. Exercise training in obese older adults prevents increase in bone turnover and attenuates decrease in hip bone mineral density induced by weight loss despite decline in bone-active hormones. *J Bone Miner Res.* 2011;26:2851–2859

45. Villareal DT, Aguirre L, Gurney AB, et al. Aerobic or resistance exercise, or both, in dieting obese older adults. *N Engl J Med.* 2017;376:1943–1955.

46. Gillespie LD, Gillespie WJ, Robertson MC, et al. Interventions for preventing falls in elderly people. *Cochrane Database Syst Rev.* 2003;(4):CD000340.

47. Orlando G, Balducci S, Bazzucchi I, et al. Neuromuscular dysfunction in type 2 diabetes: underlying mechanisms and effect of resistance training. *Diabetes Metab Res Rev.* 2016;32:40–50.

48. American Diabetes Association. Standards of Medical Care in Diabetes—2018. *Diabetes Care.* 2018;41(Suppl. 1):S1–S159.

49. Inoue M, Iso H, Yamamoto S, et al. Daily total physical activity level and premature death in men and women: results from a large-scale population-based cohort study in Japan (JPHC study). *Ann Epidemiol.* 2008;18:522–530.

50. Sedentary Behaviour Research Network. Letter to the Editor: Standardized use of the terms "sedentary" and "sedentary behaviours". *Appl Physiol Nutr Metab.* 2012;37:540–542.

51. Ishii S, Cauley JA, Crandall CJ, et al. Diabetes and femoral neck strength: findings from the Hip Strength Across the Menopausal Transition Study. *J Clin Endocrinol Metab.* 2012;97:190–197.

52. Russo CR, Lauretani F, Seeman E, et al. Structural adaptations to bone loss in aging men and women. *Bone.* 2006;38:112–118.

53. Balducci S, Sacchetti M, Orlando G, et al. Correlates of muscle strength in diabetes. The Study on the Assessment of Determinants of Muscle and Bone Strength Abnormalities in Diabetes (SAMBA). *Nutr Metab Cardiovasc Dis.* 2014;24:18–26.

54. Erlandson MC, Lorbergs AL, Mathur S, et al. Muscle analysis using pQCT, DXA and MRI. *Eur J Radiol.* 2016;85:1505–1511.

55. Balducci S, Zanuso S, Massarini M, et al. The Italian Diabetes and Exercise Study (IDES): Design and methods for a prospective Italian multicentre trial of intensive lifestyle intervention in people with type 2 diabetic and the metabolic Syndrome. *Nutr Metab Cardiovasc Dis.* 2008;18:585–595.

56. Balducci S; Zanuso S; Nicolucci A; et al. Effect of an intensive exercise intervention strategy on modifiable cardiovascular risk factors in type 2 diabetic subjects. A Randomized Controlled Trial: The Italian Diabetes and Exercise Study (IDES). *Arch Intern Med.* 2010;170:1794–1803.

57. Guralnik JM, Ferrucci L, Simonsick EM, et al. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med.* 1995;332:556–561.

58. Talbot LA, Musiol RJ, Witham EK, et al. Falls in young, middle-aged and older community dwelling adults: perceived cause, environmental factors and injury. *BMC Public Health.* 2005;18;5:86.

59. Washburn RA, Smith KW, Jette AM, et al. The physical activity scale for the elderly (PASE): Development and evaluation. *J Clin Epidemiol.* 1993;46:153–162.

60. Balducci S, Vulpiani MC, Pugliese L, et al. Effect of supervised exercise training on musculoskeletal symptoms and function in patients with type 2 diabetes: the Italian Diabetes Exercise Study (IDES). *Acta Diabetol.* 2014;51:647–654.

61. Stevens RJ, Kothari V, Adler AI, et al. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). *Clin Sci (Lond).* 2001;101:671–679.

62. Singer JD, Willett JB. Applied longitudinal data analysis: modeling change and event occurrence. New York, NY, USA: Oxford University Press 2003.

63. International Committee of Medical Journal Editors. Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. 2017, http://www.icmje.org/icmje-recommendations.pdf (accessed 18 October, 2018).

**Authors' contributions:** SB, FGC and GP conceived and designed the study. All the other authors made substantial contributions to specific parts of the protocol: recruitment and follow-up program (MV and LB), training program (MS, GO, GR, and SZ), imaging procedures (GA, LP, and AL), QUS and pQCT protocols (CRR, JH, and VDE), biochemical testing panel (PC), and statistical analysis plan (AN). GP drafted the manuscript; SB, FGC, MS, CRR, GA, JH, GO, GR, VDE, PC, LP, AL, MV, LB, SZ, and AN revised the manuscript critically for important intellectual content. All authors have given their final approval of the manuscript to be published.

**Funding statement:** This work is supported by the Metabolic Fitness Association, Monterotondo, Rome, Italy and, for the pilot study, by a grant from the Italian Ministry of Health (RF-2010-2310812) to GP. The funding sources have no role in design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the report for publication.

**Declaration of interest:** The authors declare no competing interests.

## Figure Legends

**Figure 1.** Study flow chart. LTPA = leisure-time physical activity; PASE = Physical Activity Scale for the Elderly; MS = musculoskeletal; DXA = dual-energy X-ray absorptiometry; QUS = quantitative ultrasound; pQCT = peripheral quantitative computed tomography.

**Figure 2.** Sequence of exercises during each supervised exercise training session. * Intensity of aerobic exercise will be adjusted according to improvements in predicted $VO_{2max}$, as recorded every 6 months. † Intensity of resistance exercise will be adjusted according to improvements in 1-RM, as recorded every 6 months; new resistance exercises will be introduced every 12 weeks to maintain patient's adherence and the velocity of execution during the concentric phase of the movement will be progressively increased to enhance muscle power. ‡ Height of jumps and amplitude of movements of weight bearing exercise will be also progressively increased. § Difficulty level of balance training will be gradually increased by performing the exercises with closed eyes, reducing the support area, changing visual fixation (e.g., head rotations), varying the centre of mass (e.g., limb raising), or adding a manual or cognitive task. $VO_{2max}$ = maximal oxygen consumption; 1-RM = one-repetition maximum.

**Table 1.** Exclusion criteria.

- Unable or unwilling to give informed consent or communicate with local study staff
- Current diagnosis of psychiatric disorder or hospitalization for depression in the past six months
- Self-reported alcohol or substance abuse within the past twelve months
- Self-reported inability to walk two blocks
- Musculoskeletal disorders or deformities that may interfere with participation in the intervention
- History of central nervous dysfunction such as hemiparesis, myelopathies, cerebral ataxia
- Clinical evidence of vestibular dysfunction
- Postural hypotension defined as a fall in BP when changing position of >20 mmHg (systole) or >10 mmHg (diastole)
- Cancer requiring treatment in the past five years, except for cancers that have clearly been cured or in the opinion of the investigator carry an excellent prognosis (e.g., stage 1 cervical cancer)
- Chronic obstructive pulmonary disease
- End-stage liver disease
- Chronic diabetic complications:
  - recent major acute cardiovascular event, including heart attack, stroke/transient ischemic attack(s), revascularization procedure, or participation in a cardiac rehabilitation program within the past three months
  - pre-proliferative and proliferative retinopathy
  - macroalbuminuria and/or eGFR < 45 ml/min/1.73 $m^2$
  - severe motor and sensory neuropathy
  - diabetic foot with history of ulcer
- Cardiovascular disease at cardiologic examination:
  - history of cardiac arrest
  - history of pulmonary embolism in the past six months
  - unstable angina pectoris or angina pectoris at rest
  - resting HR <45 beats/min or >100 beats/min
  - complex ventricular arrhythmia at rest or with exercise
  - uncontrolled atrial fibrillation (HR $\geq$100 beats/min)
  - NYHA Class III or IV congestive heart failure
  - acute myocarditis, pericarditis or hypertrophic myocardiopathy
  - left bundle branch block or cardiac pacemaker

- ECG treadmill test suggestive of myocardial ischemia

- Poor glycaemic and blood pressure (BP) control
  - haemoglobin (Hb) $A_{1c}$ >9.0%
  - BP >150/90 mmHg

- Bone abnormalities
  - vitamin D <10 ng/ml
  - treatment with anti-fracturative agents, estrogens, aromatase inhibitors, testosterone, corticosteroids and/or glitazones
  - previous documented non-traumatic fractures
  - SDI >3 (and >2 in a single vertebra)
  - T score ≤-2.5 at spine/hip at DXA

- Conditions not specifically mentioned above at the discretion of the clinical site

---

BP = blood pressure; eGFR = estimated glomerular filtration rate; HR = heart rate; NYHA = New York Heart Association; ECG = electrocardiogram; SDI = total spine deformity index; DXA = dual-energy X-ray absorptiometry. Subjects with $HbA_{1c}$ or BP above the indicated threshold will be receive appropriate treatment and will be re-evaluated after 3 months. Patients with vitamin D levels < 10 ng/dl will be treated with cholecalciferol 25.000 IU/week for 6 weeks and will be re-evaluated 2 weeks after the last dose.

**Table 2.** Methods for measurements of markers of bone turnover.

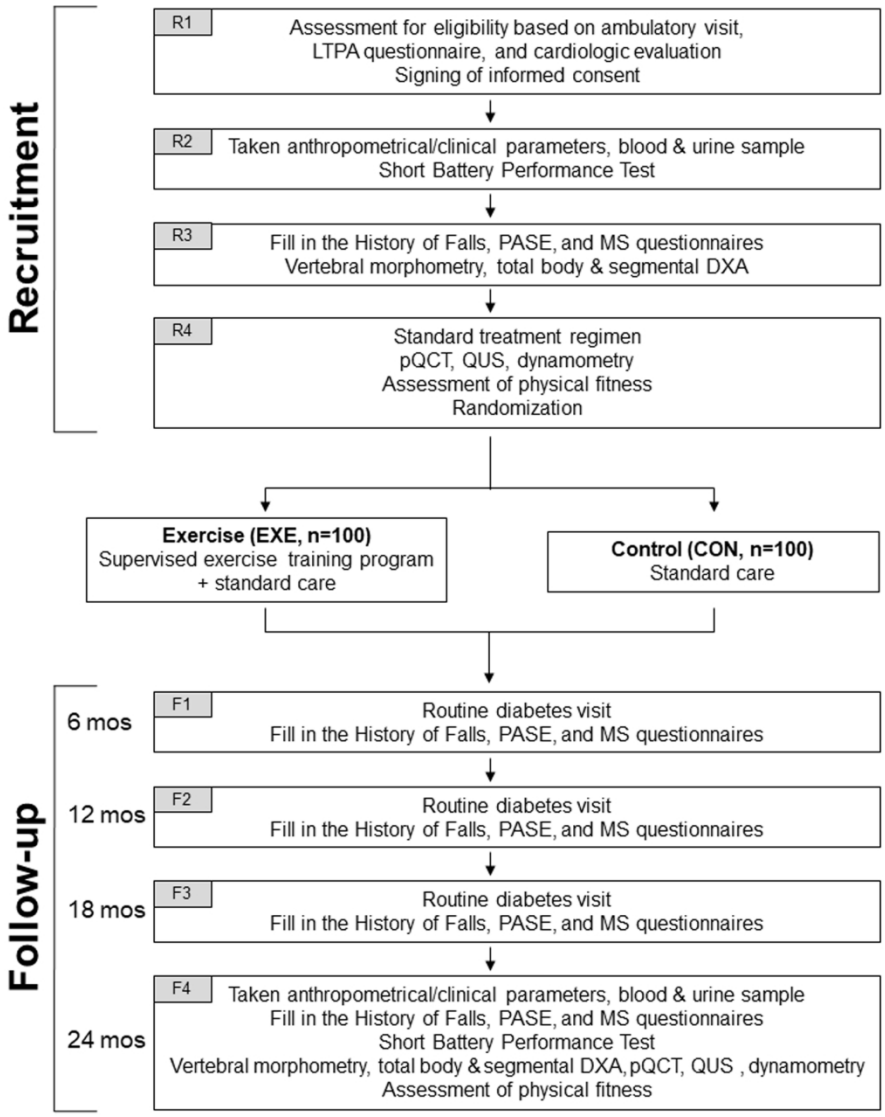
| Analyte | Method | Manifacturer |
|---|---|---|
| **Ca** | Colorimetric spectrophotometric | Architect, Abbot Diagnostics, Lake Forest, IL, USA |
| **P** | Colorimetric spectrophotometric | Architect, Abbot Diagnostics, Lake Forest, IL, USA |
| **25OH Vitamin D** | Competitive ECLIA | Liaison, DiaSorin SpA, Saluggia, Italy |
| **PTH** | ECLIA | Liaison, DiaSorin SpA, Saluggia, Italy |
| **Total ALP** | Colorimetric spectrophotometric | Architect, Abbot Diagnostics, Lake Forest, IL, USA |
| **Bone-specific ALP** | ECLIA | Liaison, DiaSorin SpA, Saluggia, Italy |
| **Osteocalcin** | ELISA | RayBiotech, Norcross, GA, USA |
| **PINP** | ELISA | RayBiotech, Norcross, GA, USA |
| **CTX-1** | ELISA | RayBiotech, Norcross, GA, USA |
| **TRAcP 5b** | ELISA | RayBiotech, Norcross, GA, USA |
| **Sclerostin** | ELISA | RayBiotech, Norcross, GA, USA |
| **DKK-1** | ELISA | RayBiotech, Norcross, GA, USA |

Ca = calcium; P = phosphorus; PTH = parathyroid hormone; ALP = alkaline phosphatase; PINP = procollagen I intact N-terminal; CTX-1 = C-terminal telopeptide of type I collagen; TRAcP 5b = tartrate-resistant acid phosphatase 5b; DKK-1= Dickkopf-1; ECLIA = chemiluminescent immunoassay; ELISA; enzyme-linked immunosorbent assay.

**Table 3.** Methods for measurements of cardiovascular risk factors.

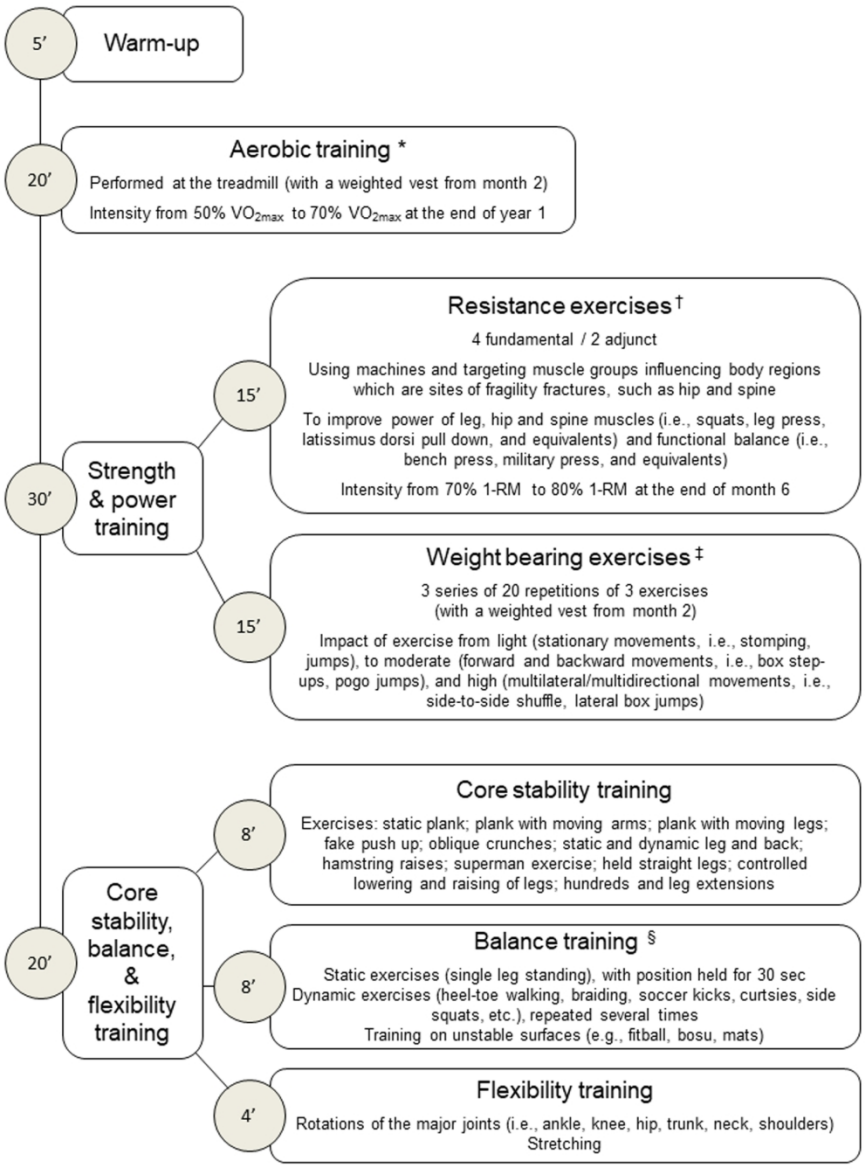
| Analyte | Method | Manifacturer |
|---|---|---|
| **$HbA_{1c}$** | HPLC (Adams TMA1C HA-8160) | Menarini Diagnostics, Florence, Italy |
| **FPG** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Triglycerides** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Total cholesterol** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **HDL cholesterol** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **hs-CRP** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Blood count** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Uric acid** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Serum creatinine** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Urinary albumin** | mAlb VITROS | Ortho Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Urinary creatinine** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |

$HbA_{1c}$ = haemoglobin $A_{1c}$; HPLC = high-performance liquid chromatography; FPG = fasting plasma glucose; hs-CRP= high sensitivity-C-reactive protein. LDL cholesterol will be calculated using the Friedewald formula (https://www.mdcalc.com/ldl-calculated), whereas glomerular filtration rate (GFR) will be estimated from serum creatinine by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (http://www.qxmd.com/calculate-online/nephrology/ckd-epi-egfr).




Study flow chart. LTPA = leisure-time physical activity; PASE = Physical Activity Scale for the Elderly; MS = musculoskeletal; DXA = dual-energy X-ray absorptiometry; QUS = quantitative ultrasound; pQCT = peripheral quantitative computed tomography.

142x189mm (300 x 300 DPI)




Sequence of exercises during each supervised exercise training session. * Intensity of aerobic exercise will be adjusted according to improvements in predicted VO2max, as recorded every 6 months. † Intensity of resistance exercise will be adjusted according to improvements in 1-RM, as recorded every 6 months; new resistance exercises will be introduced every 12 weeks to maintain patient's adherence and the velocity of execution during the concentric phase of the movement will be progressively increased to enhance muscle power. ‡ Height of jumps and amplitude of movements of weight bearing exercise will be also progressively increased. § Difficulty level of balance training will be gradually increased by performing the exercises with closed eyes, reducing the support area, changing visual fixation (e.g., head rotations), varying the centre of mass (e.g., limb raising), or adding a manual or cognitive task. $VO_{2max}$ = maximal oxygen consumption; 1-RM = one-repetition maximum.

142x189mm (300 x 300 DPI)

## Appendix A: List of participants

**Recruitment and follow-up:** Diabetes Unit, Sant'Andrea Hospital, Rome, Italy: Giuseppe Pugliese, Stefano Balducci, Martina Vitale, Tiziana Cirrito, Lucilla Bollanti, Francesco G. Conti.

**Supervised exercise training:** Metabolic Fitness Association, Monterotondo, Rome, Italy: Stefano Balducci, Gianluca Balducci, Enza Spinelli.

**DXA and vertebral morphometry evaluation:** Radiology Unit, Sant'Andrea University Hospital, Rome, Italy Giuseppe Argento, Luca Pugliese, Andrea Laghi.

**QUS and pQCT evaluation:** Diabetes Unit, Sant'Andrea Hospital, Rome, Italy: Cosimo R. Russo, Jonida Haxhi, Valeria D'Errico.

**Physical Fitness evaluation:** Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy: Massimo Sacchetti, Giorgio Orlando, Olimpia Andreani; Diabetes Unit, Sant'Andrea Hospital, Rome, Italy: Gianvito Rapisarda, Eugenio Santacroce.

**Questionnaire evaluation:** Centre for Applied Biological & Exercise Sciences, Faculty of Health & Life Sciences, Coventry University, Coventry, UK: Silvano Zanuso.

**Laboratory testing:** Laboratory of Clinical Chemistry, Sant'Andrea Hospital, Rome, Italy: Patrizia Cardelli, Gerardo Salerno, Stefano Cavallo.

**Statistical Analysis:** Centre for Outcomes Research and Clinical Epidemiology (CORE), Pescara, Italy: Antonio Nicolucci, Giuseppe Lucisano.

**Steering Committee:** Giuseppe Pugliese, Stefano Balducci, Francesco G. Conti, Massimo Sacchetti, Cosimo R, Russo, Giuseppe Argento, Silvano Zanuso, Patrizia Cardelli, Antonio Nicolucci.

## Appendix B: Questionnaires

**B1: History of Falls questionnaire**

**B2: Physical Activity Scale for the Elderly (PASE) questionnaire**

**B3: Self-report questionnaire for musculoskeletal (MS) symptoms**

## B1: History of Falls questionnaire

| | |
|---|---|
| **A. Activities prior to falling** | |
| 1. Ambulation | |
| 2. Transferring | |
| 3. Running | |
| 4. Sports | |
| 5. Stairs/curb | |
| 6. Other | |
| **B. Perceived causes (accident/environmental-related)** | |
| 1. Collapse episode | |
| 2. Dizziness/vertigo | |
| 3. Balance/gait impairment | |
| 4. Other | |
| **C. Perceived causes (environmental factors)** | |
| 1. Wet surface | |
| 2. Uneven surface/steps | |
| 3. Objects on surface/rugs | |
| 4. External forces | |
| 5. Icy surfaces | |
| 6. Other | |
| **D. Injuries sustained from fall** | |
| 1. Fractures | |
| 2. Treated injury | |
| 3. Untreated injury | |
| 4. No injury | |

## B2: Physical Activity Scale for the Elderly (PASE) questionnaire

**Q1.** Over the past 7 days, how often did you participate in sitting activities such as reading, watching TV, or doing handcrafts?

**Q1b.** On average, how many hours per day did you engage in these sitting activities?

**Q2.** Over the past 7 days, how often did you take a walk outside your home or yard for any reason? For example, for fun or exercise, walking to work, walking the dog, walking in a mall, etc?

**Q2a.** On average, how many hours per day did you spend walking?

**Q3.** Over the past 7 days, how often did you engage in light sport or recreational activities such as bowling, golf with a cart, shuffleboard, fishing from a boat or pier or other similar activities?

**Q3b.** On average, how many hours per day did you engage in these light sport or recreational activities?

**Q4.** Over the past 7 days, how often did you engage in moderate sport or recreational activities such as doubles tennis, ballroom dancing, hunting, ice skating, golf without a cart, softball or other similar activities?

**Q4b.** On average, how many hours per day did you engage in these moderate sport or recreational activities?

**Q5.** Over the past 7 days, how often did you engage in strenuous sport or recreational activities such as jogging, swimming, cycling, singles tennis, aerobic dance, skiing (downhill or cross country or other similar activities?

**Q5b.** On average, how many hours per day did you engage in these strenuous activities?

**Q6.** Over the past 7 days, how often did you do any exercises specifically to increase muscle strength or endurance, such as lifting weights or pushups, etc?

**Q6a.** On average, how many hours per day did you engage in exercises to increase muscle strength or endurance, such as lifting weights, pushups, or physical therapy with weights, etc.?

**Q7.** During the past 7 days, have you done any light housework, such as dusting, washing or drying dishes, or ironing?

**Q8.** During the past 7 days, have you done any heavy housework or chores such as vacuuming, scrubbing floors, washing windows, or carrying wood?

**Q9a.** During the past 7 days, did you engage in home repairs like painting, wallpapering, electrical work, etc.?

**Q9b.** During the past 7 days, did you engage in lawn work or yard care, including snow or leaf removal, chopping wood, etc?

**Q9c.** During the past 7 days, did you engage in outdoor gardening?

**Q9d.** During the past 7 days, did you engage in caring for another person such as a child, dependent spouse, or another adult?

**Q10.** During the past 7 days, did you work for pay or as a volunteer?

**Q10a.** How many hours per week did you work for pay and/or as a volunteer?

**Q10b.** Which of the following categories best describes the amount of physical activity required on your job and/or volunteer work?

Category 1 ("Mainly sitting with slight arm movements") includes examples such as: office worker, watchmaker, seated assembly line worker, bus driver, etc.

Category 2 ("Sitting or standing with some walking") includes examples such as: cashier, general office worker, light tool and machinery worker.

Category 3 ("Walking, with some handling of materials generally weighing less than 50 pounds") includes examples such as: mailman, waiter/waitress, construction worker, heavy tool and machinery worker.

Category 4 ("Walking and heavy manual work often requiring handling of materials weighing over 50 pounds") includes examples such as: lumberjack, stonemason, farm or general labourer].

## B3. Self-reported questionnaire for MS symptoms

| | |
|---|---|
| **SHOULDER** | |
| **1** | Do you have pain during rotation of the arm? |
| **2** | Are you awakened by pain during the night? |
| **3** | Do you have pain on reaching objects above the head? |
| **4** | Do you have pain on lifting objects? |
| **5** | Do you have pain or soreness upon awakening that passes later on during the day? |
| **6** | Have you taken anti-inflammation drugs or pain-killers? |
| **ARM** | |
| **7** | Do you feel that you have less strength? |
| **8** | Dou you feel that one arm is weaker than the other? |
| **9** | Do you have pain at the maximum extension of the forearm? |
| **ELBOW** | |
| **10** | Do you have pain on lifting an object? |
| **11** | Do you have pain on hitting against a rigid object? |
| **12** | Have you taken anti-inflammation drugs or pain-killers? |
| **WRIST** | |
| **13** | Do you have pain on lifting an object? |
| **14** | Do you have pain on hitting against a rigid object? |
| **15** | Have you taken anti-inflammation drugs or pain-killers? |
| **HAND:** Do you feel "pins and needles"? If so, in which finger? | |
| **16** | I |
| **17** | II |
| **18** | III |
| **19** | IV |
| **20** | V |
| **SPINE: THORACO-CERVICAL** | |
| **21** | Do you have pain/tenderness/ pins & needles on turning your head from side to side? |
| **22** | Do you often have pain or headache or heaviness of the head or neck? |
| **23** | Do you have pain between the shoulder blades? |
| **24** | Do you feel it necessary to move your head from side to side to get moving and feel? |
| **25** | Do you have episodes of painful sudden acute stiffness of the neck? |
| **26** | Have you taken anti-inflammation drugs or pain-killers? |
| **SPINE: LUMBO-SACRAL** | |
| **27** | Do you have pain on bending to tie your shoe laces? |
| **28** | Do you have any back-pain on turning left or right? |
| **29** | Do you have a feeling of heaviness in your back on standing for long hours? |
| **30** | Do you have bothersome feeling when sitting still? Do you have to get up? |
| **31** | Did you have one episode of sudden intense back pain that leaves you unable to move? |
| **32** | Have you taken anti-inflammation drugs or pain-killers? |

| | |
|---|---|
| **HIP** | |
| **33** | Do you have pain on crossing your legs? |
| **34** | Do you have any pain when opening your legs to the maximum? |
| **35** | Do you often have pain from your buttocks along the length of the leg down to your ankles? |
| **36** | Have you taken a single dose of anti-inflammatory drugs or pain-killers? |
| **KNEE** | |
| **37** | Do you have pain in the knee in the act of sitting down or getting up? |
| **38** | Do you have pain in your knee after having walked a lot? |
| **39** | Is your knee often swollen at the end of the day? |
| **40** | Do you have pain in the "good" knee? |
| **41** | Do you have pain or a bothersome feeling as you kneel down? |
| **42** | When lying in bed, do you feel the need to move your legs, ones or more than once? |
| **43** | Have you taken a single dose of anti-inflammatory drugs or pain-killers? |
| **FOOT** | |
| **44** | Do you often feel a sensation of pins and needles that runs down to one or more toes? |
| **45** | Do you have any difficulty in standing on your toes? |
| **46** | Do you have any pain in your foot after walking for a long time? |
| **47** | Do you have pain on taking the first step in the morning? |
| **48** | Do you have any difficulty or pain when putting on stiff orthopaedic shoes? |
| **49** | Do you have pain under the heel when walking a lot? |
| **50** | Have you taken a single dose of anti-inflammatory drugs or pain-killers? |

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

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

| Section/item | Item No | Description | Addressed on page number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 4, 17 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | Appendix D |
| Protocol version | 3 | Date and version identifier | 17 |
| Funding | 4 | Sources and types of financial, material, and other support | 24 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1, 24 |
| | 5b | Name and contact information for the trial sponsor | 24, Appendix D |
| | 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 | 24 |
| | 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) | Appendix A |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 5-7 |
| | 6b | Explanation for choice of comparators | NA |

| | | | |
|---|---|---|---|
| Objectives | 7 | Specific objectives or hypotheses | 7 |
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 8 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 9, Appendix A |
| 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) | 8, Table 1 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 11-12, Fig. 2 |
| | 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) | NA |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | 9 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 12 |
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 12 |
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 9-11, Fig. 1 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 15-16 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 9 |

**Methods: Assignment of interventions (for controlled trials)**

Allocation:

| | | | |
|---|---|---|---|
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 10 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 10 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 10 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 10, 12 |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | NA |

**Methods: Data collection, management, and analysis**

| | | | |
|---|---|---|---|
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 12-15, Tables 2-3, Appendix B |
| | 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 | 9 |
| 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 | 15 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 15, 16 |

| | | | |
|---|---|---|---|
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 16 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 16 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | NA |
| | 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 | NA |
| 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 | 15 |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | NA |
| **Ethics and dissemination** | | | |
| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 17 |
| 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) | 17 |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 9 |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | NA |
| 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 | 15 |

| | | | |
|---|---|---|---|
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 24 |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 15 |
| 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 | 15 |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 17 |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | 17 |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 17 |
| **Appendices** | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Appendix E |
| 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 | NA |

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

## Appendix D: World Health Organization Trial Registration Data Set

1. **Primary Registry and Trial Identifying Number:** ClinicalTrials.gov; NCT02421393; URL https://clinicaltrials.gov/ct2/show/NCT02421393.
2. **Date of Registration in Primary Registry:** 20 April. 2014
3. **Secondary Identifying Numbers:** NA.
4. **Source(s) of Monetary or Material Support:** Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: info@metabolicfitness.it.
5. **Primary Sponsor:** Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: info@metabolicfitness.it.
6. **Secondary Sponsor(s):** NA.
7. **Contact for Public Queries: Stefano Balducci, MD,** Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: sbalducci@esinet.it.
8. **Contact for Scientific Queries: Giuseppe Pugliese,** M.D., Ph.D., Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, Via di Grottarossa, 1035-1039 - 00189 Rome, Italy; Phone: +39-0633775440; Fax: +39-0633776327; E-mail: giuseppe.pugliese@uniroma1.it.
9. **Public Title:** The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes.
10. **Scientific Title:** The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes.
11. **Countries of Recruitment:** Italy.
12. **Health Condition(s) or Problem(s) Studied:** type 2 diabetes (T2D).
13. **Intervention:**
    a. Intervention arm
       - Name: Supervised exercise training.
       - Description: two weekly supervised mixed exercise training sessions for two years, on top of standard care.
    b. Standard care.
14. **Key Inclusion and Exclusion Criteria**
    a. Inclusion criteria: known T2D (defined by the ADA criteria) of at least 1-year duration. Additional requirements are age 40-80 years; BMI 27-40 kg/m$^2$; sedentary lifestyle (i.e., more than 8 hours/day spent in any waking behaviour characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture) and physically inactivity (i.e., insufficient amounts of PA according to current guidelines) from at least 6 months; a Short Battery Performance Test score ≥4; ability to walk 1.6 Km without assistance; and eligibility after cardiologic evaluation.
    b. Exclusion criteria: unable or unwilling to give informed consent or communicate with local study staff; current diagnosis of psychiatric disorder or hospitalization for depression in the past six months; self-reported alcohol or substance abuse within the past twelve months; self-reported

inability to walk two blocks; musculoskeletal disorders or deformities that may interfere with participation in the intervention; history of central nervous dysfunction such as hemiparesis; myelopathies; cerebral ataxia; clinical evidence of vestibular dysfunction; postural hypotension defined as a fall in BP when changing position of >20 mmHg (systole) or >10 mmHg (diastole); currently pregnant or nursing; cancer requiring treatment in the past five years, except for cancers that have clearly been cured or in the opinion of the investigator carry an excellent prognosis (e.g., stage 1 cervical cancer); chronic obstructive pulmonary disease; end-stage liver disease; chronic diabetic complications (recent major acute cardiovascular event, including heart attack, stroke/transient ischemic attack(s), revascularization procedure, or participation in a cardiac rehabilitation program within the past three months; pre-proliferative and proliferative retinopathy; macroalbuminuria and/or eGFR < 45 ml/min/1.73 $m^2$; severe motor and sensory neuropathy; diabetic foot with history of ulcer); cardiovascular disease at cardiologic examination (history of cardiac arrest; history of pulmonary embolism in the past six months; unstable angina pectoris or angina pectoris at rest; resting HR <45 beats/min or >100 beats/min; complex ventricular arrhythmia at rest or with exercise; uncontrolled atrial fibrillation with HR >100 beats/min; NYHA Class III or IV congestive heart failure; acute myocarditis; pericarditis or hypertrophic myocardiopathy; left bundle branch block or cardiac pacemaker); treatment with anti-fracture agents, oestrogens, aromatase inhibitors, testosterone, corticosteroids and/or glitazones; previous documented non-traumatic fractures; spinal deformity index (SDI) >3 (and >2 in a single vertebra); and a T score <-2.5 at spine/hip at DXA; haemoglobin (Hb) $A_{1c}$ >9.0%; blood pressure (BP) >150/90 mmHg; vitamin D <10 ng/ml; conditions not specifically mentioned above at the discretion of the clinical site.

15. **Study Type**

    a. Type of study: interventional.

    b. Study design:

    - Method of allocation: randomized
    - Masking: no (assessor-blinded)
    - Assignment: parallel
    - Purpose: testing the efficacy of a specific exercise training program in improving bone quality and strength in patients with T2D

    c. Phase: NA

    d. Allocation concealment mechanism and sequence generation: centralized randomization stratified by age, gender, and type of diabetes treatment (non-insulin versus insulin therapy), using a permuted-block randomization software which randomly varies the block size.

15. **Date of First Enrolment:** November 1, 2018 (expected).

16. **Target Sample Size:** 200

17. **Recruitment Status: recruiting.**

16. **Primary Outcome(s)**

- Name: baseline to end-of-study change in Trabecular Bone Score (TBS);
- Method of measurement: spine dual-energy X-ray absorptiometry (DXA)-derived software-based measure;
- Time points: baseline and end-of-study.

17. **Key Secondary Outcomes**

a. Name: baseline to end-of-study change in broadband ultrasound attenuation (BUA), speed of sound (SOS), and quantitative ultrasound index (QUI); methods of measurement: quantitative ultrasound (QUS); time points: baseline and end-of-study.

b. Name: baseline to end-of-study change in multiple measured and calculated bone parameters; methods of measurement: peripheral quantitative computed tomography (pQCT); time points: baseline and end-of-study.

c. Name: baseline to end-of-study change in bone mineral density (BMD) and other DEXA-derived measures; method of measurement: spine and hip DXA; time points: baseline and end-of-study.

d. Name: baseline to end-of-study change in markers of bone turnover; method of measurement: immunochemical methods; time points: baseline and end-of-study.

e. Name: baseline to end-of-study change in body composition; method of measurement: total body DXA; time points: baseline and end-of-study.

f. Name: baseline to end-of-study change in muscle strength; methods of measurement: isometric muscle strength test; time points: baseline and end-of-study.

g. Name: baseline to end-of-study change in muscle cross-sectional area; method of measurement: pQCT; time points: baseline and end-of-study.

h. Name: baseline to end-of-study change in balance, gait and power; method of measurement: Short Battery Performance Test; time points: baseline and end-of-study.

i. Name: number of falls; 17-item History of Falls questionnaire; time points: baseline and every 6 months thereafter for 7 years (2-year trial + 5-year post-trial follow-up).

j. Name: symptomatic fractures; method of measurement: clinical and radiographic records; time points: baseline and every 6 months thereafter for 7 years (2-year trial + 5-year post-trial follow-up).

k. Name: asymptomatic and symptomatic fractures; method of measurement: vertebral morphometry; time points: baseline and every 6 months thereafter for 7 years (2-year trial + 5-year post-trial follow-up).

## Appendix E: Informed consent

### Patient Information Sheet

### TITLE OF THE STUDY: The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes

This study is registered at ClinicalTrial.gov as "Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET-BONE) in type 2 diabetes: an exercise intervention program for reducing the risk of fractures "(N. NCT02421393, URL https://clinicaltrials.gov/ct2/show/NCT02421393)

**IDENTIFICATION OF THE STUDY**

Dear Sir/Madam,

The study, which your physician (diabetes specialist) is inviting you to participate in, aims to evaluate the effect of 2-year training consisting of supervised and combined aerobic and resistance exercise sessions in subjects with type 2 diabetes mellitus on:

- bone quality and mass;
- bone metabolism;
- muscle strength and mass;
- balance and gait;
- falls;
- symptomatic and asymptomatic fractures.

The hypothesis is that a specific exercise training program produces a significant improvement in the qualitative and quantitative bone parameters by influencing bone metabolism, with a consequent reduction in the risk of fractures and, in the long term, a significantly reduced number of fractures.

The research involves about 200 patients with type 2 diabetes.

During the study you will be assigned to one of the following two groups:

1. Exercise (EXE) group, which receives standard care and participates in two weekly mixed exercise training sessions for two years, supervised by an exercise specialist at the Metabolic Fitness Association.
2. Control (CON) group, which receives only standard care.

The study will have a duration of 2 years plus a 5-year post-trial follow-up, during which you will be subjected to 6-month monitoring visits. The parameters reported above will be assessed at the beginning and at the end of the 2-year period, except for falls and fractures, which will be assessed every 6 months for the entire 7 year period (2-year trial + 5-year post-trial follow-up).

**PROTECTION OF PERSONAL DATA:**

- All information concerning you, the collection and processing of which is connected and indispensable to the achievement of the objectives of this study, will be treated in a manner suitable to ensure

absolute confidentiality and security in accordance with the provisions for the protection of personal data and the right to privacy (Italian Data Protection Act, No. 675 of December 6, 1996 and subsequent amendments/additions).

- You will be identified by a code and the clinical information concerning you will not be disclosed without your written permission. The data collected will consist of your initials, date of birth, sex and otherwise sensitive clinical data as suitable to reveal your state of health.
- As a participant in the processing of your personal data, you will have full access, through your family doctor, to the information concerning you. You will also have the right to exercise all the rights of cancellation, transformation, integration, updating, correction and blocking of your data within the limits set out in art. 13 of the Italian Data Protection Act 675/96 mentioned above. You will not be charged any fee for the scheduled exams, the results of which will be promptly communicated to your family doctor.

**STUDY BENEFITS:**

- Upon agreeing to participate in this study, you might be assigned to follow a supervised exercise training program. Whatever group you are assigned to, you will be under strict control by a staff-member, medical or otherwise, specialized in the management of type 2 diabetes, including physical activity/exercise therapy.
- In addition, your doctor may become aware of the presence of cardiovascular risk factors or complications to be monitored.
- Finally, the knowledge acquired thanks to your participation will be useful both for you and for other patients.

**PARTICIPATION IN THE STUDY:**

- Your participation in this study is completely cost-free and, if you decide not to take part, you will still be assisted in the most appropriate medical treatment.
- We invite you to ask your family physician any question you deem appropriate. Your doctor will also ask you to sign and date the consent form for the processing of personal data to confirm that you have read all the information contained herein, which includes that you have understood the aims of the study and most importantly, that you have freely given your consent to the collection and processing of your personal data.

## Patient Consent Form

**TITLE OF THE STUDY: The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes**

I, the undersigned _____ born in _____ on _____

and resident in _____

hereby declare, after reading the information, the following:

- to have read and understood the patient information sheet of the aforementioned study and to have had ample time and opportunity to ask questions and obtain satisfactory answers to the investigator;
- to have understood that my participation is voluntary and that I can withdraw from the study at any time, without having to explain or influence any future medical assistance in any way;
- to have understood that my personal data will be processed according to the regulations in force specified in the information sheet of the study and that I can exercise my rights by contacting the Data Controller at any time and in the manner specified in accordance with art. 7, Legislative Decree n. 196 of 30/06/2003, (so-called Privacy Code).

Following these statements, I declare that I, the undersigned, freely:

- accept to participate in the study mentioned above;
- consent to the processing of personal and sensitive data collected in the context of this study, in the terms and methods indicated and explained in the information, aware that anonymity in the treatment will be guaranteed;
- consent that the investigator and his collaborators, as expressly indicated in the informative report, collect and process the data deriving from the investigations for the express purpose of a scientific publication.

**Signature of the patient** _____ **date** _____

**Surname and name of the patient** _____

**Signature of the investigator** _____ **date** _____

**Surname and name of the investigator** _____

BMJ Open

# Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes: an exercise intervention program for reducing the risk of fractures.
# Study protocol for a randomized clinical trial



| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2018-027429.R1 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 24-Apr-2019 |
| Complete List of Authors: | Balducci, Stefano; La Sapienza University, Department of Clinical and Molecular Medicine<br>Conti, Francesco; La Sapienza University, Rome, Department of Clinical and Molecular Medicine<br>Sacchetti, Massimo; Foro Italico University, Department of Human Movement and Sport Sciences<br>Russo, Cosimo; Metagym<br>Argento, Giuseppe; Sant'Andrea University Hospital, Radiology Unit<br>Haxhi, Jonida; La Sapienza University, Department of Clinical and Molecular Medicine<br>Orlando, Giorgio; Foro Italico University, Department of Human Movement and Sport Sciences<br>Rapisarda, Gianvito; La Sapienza University, Department of Clinical and Molecular Medicine<br>D'Errico, Valeria; La Sapienza University, Department of Clinical and Molecular Medicine<br>Cardelli, Patrizia; La Sapienza University, Department of Clinical and Molecular Medicine<br>Pugliese, Luca; Sant'Andrea University Hospital, Radiology Unit<br>Laghi, Andrea; Sant'Andrea University Hospital, Radiology Unit<br>Vitale, Martina; La Sapienza University, Department of Clinical and Molecular Medicine<br>Bollanti, Lucilla; La Sapienza University, Department of Clinical and Molecular Medicine<br>Zanuso , S; Coventry University, Centre for Applied Biological & Exercise Sciences<br>Nicolucci, Antonio; Center for Outcomes Research and clinical Epidemiology (CORE), Pescara, Italy.,<br>Pugliese, Giuseppe; La Sapienza University, Rome, Department of Clinical and Molecular Medicine |
| Primary Subject Heading: | Diabetes and endocrinology |
| Secondary Subject Heading: | Sports and exercise medicine |
| Keywords: | General diabetes < DIABETES & ENDOCRINOLOGY, Diabetes & |

| | |
|---|---|
| | endocrinology < INTERNAL MEDICINE, Bone diseases < ORTHOPAEDIC & TRAUMA SURGERY, PREVENTIVE MEDICINE |
| | |

# Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes: an exercise intervention program for reducing the risk of fractures

## Study protocol for a randomized clinical trial

Stefano Balducci [1,2,3], Francesco G. Conti [1,2], Massimo Sacchetti [4], Cosimo R. Russo [5], Giuseppe Argento [6], Jonida Haxhi [2,3,4], Giorgio Orlando [4*], Gianvito Rapisarda [2,3], Valeria D'Errico [2,3], Patrizia Cardelli [1,7], Luca Pugliese [6†], Andrea Laghi [6,8], Martina Vitale [1,2], Lucilla Bollanti [1,2], Silvano Zanuso [9], Antonio Nicolucci [10], and Giuseppe Pugliese [1,2]; for the SWEET BONE Investigators [‡].

[1] Department of Clinical and Molecular Medicine, ''La Sapienza'' University, Rome, Italy; [2] Diabetes Unit, Sant'Andrea University Hospital, Rome, Italy; [3] Metabolic Fitness Association, Monterotondo, Rome, Italy; [4] Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy; [5] Metagym Fitness Centre, Florence, Italy; [6] Radiology Unit, Sant'Andrea University Hospital, Rome, Italy; [7] Laboratory of Clinical Chemistry, Sant'Andrea University Hospital, Rome, Italy; [8] Department of Radiological Sciences, Oncology and Pathology, ''La Sapienza'' University, Rome, Italy; [9] Centre for Applied Biological & Exercise Sciences, Faculty of Health & Life Sciences, Coventry University, Coventry, UK; and [10] Centre for Outcomes Research and Clinical Epidemiology (CORESEARCH), Pescara, Italy.

Current address: [*] School of Healthcare Science, Faculty of Science & Engineering, Manchester Metropolitan University, Manchester, UK; [†] Department of Diagnostic Imaging, "Tor Vergata" University, Rome, Italy.

[‡] See Appendix A for the complete list of the SWEET_BONE Investigators.

**Corresponding Author:** Giuseppe PUGLIESE, MD, PhD, Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, Via di Grottarossa, 1035-1039 - 00189 Rome, Italy; Phone: +39-0633775440; Fax: +39-0633776327; E-mail: giuseppe.pugliese@uniroma1.it.

**Key words:** type 2 diabetes; bone quality; bone mass; bone fractures; exercise; physical fitness.

**Word count:** abstract 300; text 4,000.

## Strengths and limitations of this study

- This is the first study investigating whether a specifically designed exercise training program of 2-year duration is effective in improving bone quality and strength in patients with type 2 diabetes, thus reducing the increased fracture risk characterizing these individuals.
- A wide range of parameters of bone quality and strength is assessed, together with measures of bone mass and muscle mass, strength and power, which all may affect fracture risk.
- All the physicians, exercise specialists, and outcome assessors have been specifically trained for conducting this trial and participated in a pilot study aimed at setting up the trial protocol.
- The efficacy of the intervention in reducing falls and fractures will be assessed over an extended 7-year period, including a 5-year post-intervention follow-up.
- Generalizability and implementation in clinical practice of this approach will require further investigation and validation in different cohorts or contexts.

## Abstract

**Introduction.** Type 2 diabetes (T2D) is associated with an increased fracture risk despite normal-to-increased bone mineral density, suggesting reduced bone quality. Exercise may be effective in reducing fracture risk by ameliorating muscle dysfunction and reducing risk of fall, though it is unclear whether it can improve bone quality.

**Methods and analysis.** The "Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in T2D" is an open-label, assessor-blinded, randomized clinical trial comparing an exercise training program of 2-year duration, specifically designed for improving bone quality and strength, with standard care in T2D individuals. Two-hundred T2D patients aged 65-75 years will be randomized 1:1 to supervised exercise training or standard care, stratified by gender, age ≤ or >70 years, and non-insulin or insulin treatment. The intervention consists of two weekly supervised sessions, each starting with 5 min of warm-up, followed by 20 min of aerobic training, 30 min of resistance training, and 20 min of core stability, balance, and flexibility training. Participants will wear weighted vests during aerobic and resistance training. The primary endpoint is baseline to end-of-study change in trabecular bone score, a parameter of bone quality consistently shown to be reduced in T2D. Secondary endpoints include changes in other potential measures of bone quality, as assessed by quantitative ultrasound and peripheral quantitative computed tomography; bone mass; markers of bone turnover; muscle strength, mass, and power; balance and gait. Falls and asymptomatic and symptomatic fractures will be evaluated over 7 years, including a 5-year post-trial follow-up. The superiority of the intervention will be assessed by comparing between-groups baseline to end-of-study changes.

**Ethics and dissemination.** This study was approved by the institutional ethics committee. Written informed consent will be obtained from all participants. The study results will be submitted for peer-reviewed publication.

**Registration details.** ClinicalTrials.gov, NCT02421393.

## Introduction

Risk of fracture is significantly increased in type 1 diabetes (T1D) and, to a lower extent, in type 2 diabetes (T2D).[1, 2] Nevertheless, bone mineral density (BMD) was reported to be normal or even increased in T2D patients, whereas it was found almost consistently reduced in T1D individuals.[1] Notably, in T2D patients, the increase in fracture risk remained after adjustment for BMD [3-5] and also for falls,[3, 4, 6] which are more frequent in older individuals with T2D than in those without.[7] In addition, as compared with non-diabetic individuals, patients with T2D have a higher T-score for a similar fracture risk.[8] While the preserved bone mass may account for the lower fracture risk in T2D versus T1D, a reduced bone quality has been claimed to explain the discrepancy between normal BMD and increased fracture risk in T2D patients.[1, 2]

Bone quality is determined by (a) bone architecture, including geometry (macro-architecture) and micro-architecture; and (b) material properties, including mineralization and collagen cross-links, which in turn are influenced by bone turnover as well as by accumulation of microdamage and microstructural discontinuities such as microporosity and lamellar boundaries.[9, 10] While conventional dual-energy X-ray absorptiometry (DXA) measures bone mass, several techniques have been proposed for non-invasive assessment of bone quality.[11, 12] The trabecular bone score (TBS) is a gray-level texture measurement based on 2D projection images acquired during a DXA lumbar spine scan.[13] It was consistently found to be reduced in T2D patients with versus those without fracture [14] and in T2D versus non-diabetic individual, [15-20] and to predict fractures independently of BMD.[15] Quantitative ultrasound (QUS), usually performed at the heel, provides an estimate of BMD,[21] which predicted fracture risk better than DXA-derived BMD in older women with T2D.[22] In addition, QUS evaluates parameters of bone quality, including micro-architecture and material properties.[23, 24] However, QUS-derived bone structure measures were not consistently lower in T2D patients with versus those without fracture [25, 26] and in T2D versus non-diabetic individuals.[27, 28] In addition to volumetric BMD (vBMD), low- and high-resolution peripheral quantitative computed tomography (pQCT) provides measures of bone geometry and architecture, which are surrogates of bone quality and

strength.[11]. Higher cortical porosity and lower calculated strength were reported in T2D patients with versus those without fracture [29, 30] and in T2D versus non-diabetic individuals.[31-34]

Physical activity (PA)/exercise has been suggested as an effective tool for improving bone health in individuals at high fracture risk. It is known that both compressive loading from weight bearing and muscle contraction deform the osteocytes, which function as strain transducers by signalling osteoblasts, osteoclasts, and other cells to produce or break down bone.[35, 36] Appropriate types, amounts, and directions of strain result in bone mass maintenance, bone formation, and/or changes in bone geometry that improve bone strength.[37]

Exercise was shown to improve BMD to a relatively small, but clinically significant extent.[38] There is also a great deal of evidence from observational studies that higher PA levels are associated with fewer fractures in community-dwelling populations,[39] and postmenopausal women who performed spinal extension exercises showed a lower incidence of vertebral fractures.[40] Combination of diet and exercise was shown to provide greater improvement in physical function than either intervention alone.[41] Moreover, exercise training prevented the increase in bone turnover and attenuated the decrease in hip BMD associated with diet-induced weight loss,[42] and resistance exercise attenuated diet-induced decrease in muscle mass and BMD more than aerobic training.[43] Resistance exercise was also shown to decrease falls and risk of falls, especially when focused on strengthening the hip and ankle muscles involved in balance maintenance.[44]

These observations indicate that PA/exercise, especially of resistant type, may be effective in reducing fracture risk in T2D patients by ameliorating muscle mass, strength, and quality [45] and reducing falls and risk of fall.[7] However, it is unclear whether PA/exercise may reduce fracture risk also by directly improving bone health in individuals with preserved BMD, such as those with T2D. Indeed, to date, there are no data on whether exercise training is effective in ameliorating bone quality and whether improved quality results in increased bone strength and reduced fracture risk in patients with T2D.

The “Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in T2D” is aimed at investigating the efficacy of a specific exercise intervention program of 2-year duration on parameters of bone quality and strength in patients with T2D.

## Methods and analysis

### Trial design

The SWEET BONE is an open-label, assessor-blinded, parallel, superiority randomized clinical trial (RCT) comparing a specifically designed exercise intervention program with standard care in individuals with T2D. The trial flow chart is shown in Figure 1.

### Participants

This study will enrol patients with T2D (defined by the American Diabetes Association criteria [46]) of at least 5-year duration, of both sexes, aged 65-75 years. Additional requirements will be: physically inactivity (i.e. insufficient amounts of PA according to current guidelines) [47] and sedentary lifestyle (i.e. more than 8 hours/day spent in a sitting or reclining posture) [48] from ≥6 months; body mass index (BMI) 27-40 kg/m$^2$; ability to walk 1.6 Km without assistance; a Short Battery Performance Test score ≥4; and eligibility after cardiologic evaluation. All patients attending the Diabetes Clinic will be evaluated for eligibility.

The criteria listed in Table 1 will be used to exclude individuals with conditions limiting or contraindicating PA, affect conduct of the trial, reduce lifespan, and/or affect the safety of intervention. Among exclusion criteria there are treatment with anti-fracture agents, oestrogens, aromatase inhibitors, testosterone, corticosteroids and/or glitazones; previous documented non-traumatic fractures; spinal deformity index (SDI) >5 (and >2 in a single vertebra); and a T score <-2.5 at spine/hip at DXA. Individuals with haemoglobin (Hb) $A_{1c}$ >9.0%, blood pressure (BP) >150/90 mmHg, and/or vitamin D <10 ng/ml will be re-evaluated for eligibility after receiving appropriate glucose- and BP-lowering therapy and a 6-week treatment with cholecalciferol 25.000 IU/week, respectively.

A sample of 50 non-diabetic individuals meeting the inclusion/exclusion criteria reported above (except for T2D-related criteria) and matched 1:4 by age, gender, and BMI will serve as controls for baseline measures.

**Investigators**

All the SWEET-BONE physicians, exercise specialists, and outcome assessors (see Appendix A) have been specifically trained for conducting this RCT and participated in a pilot study aimed at setting up the trial protocol.

To minimize dropout and reduce the attrition bias due to missing data, both physicians and exercise specialists have been instructed on how to promote participant retention in the trial. In particular, they have been recommended to contact participants at regular intervals, to keep up to date contact information for participants, and to collect complete data for the primary and secondary outcomes, regardless of whether individuals continue to receive the assigned intervention.

**Recruitment**

Starting on 1 November, 2018, 200 patients will be recruited at the Diabetes Unit of Sant'Andrea University Hospital, a tertiary referral, outpatients Diabetes Clinic in Rome, Italy. All patients attending the clinic will be evaluated for eligibility. The recruitment process will include four visits designated as R1, R2, R3, and R4.

On R1, eligible patients will be identified based on medical history, clinical examination, and results of the Minnesota leisure-time PA questionnaire. Then, patients will be asked to sign an informed consent and will be registered in the SWEET-BONE database available at http://www.metabolicfitness.it/. Finally, patients will undergo a cardiologic examination, including a resting electrocardiogram (ECG) and, based on clinical judgment, an echocardiogram and/or an ECG treadmill test.

On R2, baseline anthropometrical and clinical parameters and blood and urine samples for biochemical testing will be taken. Subsequently, participants will perform a Short Battery Performance Test and undergo measurement of ankle-brachial index and fundus evaluation. Finally, patients will attend a run-in session for familiarization with testing devices and protocols for the assessment of physical fitness.

On R3, patients will be asked to fill in the History of Falls questionnaire, the Physical Activity Scale for the Elderly (PASE) questionnaire, and a questionnaire for musculoskeletal (MS) symptoms. Then, participants will

undergo x-ray of dorso-lumbar spine for vertebral morphometry, and total body and segmental DXA. Finally, they will attend another run-in session for familiarization prior to the assessment of physical fitness.

On R4, patients will receive a standard treatment regimen including nutritional therapy and prescription of pharmacological agents, as needed. Then, they will undergo the following procedures: peripheral QCT (pQCT), calcaneal QUS, and dynamometry. Finally, patients will be subjected to the assessment of physical fitness and will be informed about group assignment.

**Randomization**

Patients will be randomized 1:1 to supervised exercise training on top of standard care (exercise, EXE, group; n=100) versus standard care (control, CON, group; n=100) for 24 months.

Randomization will be stratified by gender (males versus females), age (65-70 versus 71-75 years) and type of diabetes treatment (non-insulin versus insulin), using a permuted-block randomization software which randomly varies the block size. To ensure allocation concealment, randomization will be centralized at the Centre for Outcomes Research and Clinical Epidemiology (CORESEARCH), and the group assignment of newly recruited patients will be communicated to the investigators by telephone call.

After randomization, participants, physicians and exercise specialists will not be blinded to group assignment, as blinding in unfeasible in exercise intervention studies.

**Follow-up**

Participants from both groups will attend four follow-up visits, designated as F1, F2, F3, and F4, at month 6, 12, 18, and 24, respectively.

On F1, F2, and F3, patients will undergo a routine diabetes visit, with eventual adjustment of dietary and pharmacological prescriptions, and will be asked to fill in the History of Falls, PASE, and MS questionnaires. On F2 only, intermediate anthropometrical and clinical parameters and blood and urine samples for centralized biochemical testing will be taken.

On F4, end-of study anthropometrical and clinical parameters and blood and urine samples for biochemical testing will be taken. Then, participants will be asked to fill in the History of Falls, PASE, and MS questionnaires and to perform a Short Battery Performance Test. On different days, patients will undergo x-ray of dorso-lumbar spine for vertebral morphometry, total body and segmental DXA, pQCT, calcaneal QUS, dynamometry, and assessment of physical fitness.

**Post-trial follow-up**

Participants will be followed every 6 months for additional 5 years for routine diabetes visits. On these occasions, they will be asked to provide clinical records on eventual fractures, to fill in the History of Falls, PASE, and MS questionnaires, and to perform a Short Battery Performance Test. At the end of the 5-year post-trial follow-up, participants will undergo vertebral morphometry to detect asymptomatic fractures.

**Intervention**

The training program for the EXE group will consist of two 75-min weekly sessions, supervised by an exercise specialist in the gym facility of the Metabolic Fitness Association (Figure 2). We conducted a pilot study on a small sample of T2D patients meeting the inclusion/exclusion criteria for this RCT in order to set up the training program.

Each session will start with 5 min of warm up, followed by 20 min of aerobic training using treadmill. Then, patients will perform 30 min of resistance (strength and power) training consisting of 6 resistance exercises using machines and targeting muscle groups influencing body regions which are sites of fragility fractures (15 min) and 3 20-repetition series of 3 different “weight bearing” exercises (15 min). The session will end with 20 min of core stability training (8 min), which improves the ability to control the position and movement of the central portion of the body and targets the deep abdominal muscles that assist in posture maintenance and arm and leg movements, followed by balance (8 min) and flexibility (4 min) training.

The exercise intensity and difficulty level will be increased gradually in order to ensure safety and prevent attrition, as shown in a previous RCT [49] and confirmed by the pilot study. In particular, the intensity of

aerobic and resistance exercise will be increased from light to moderate and adjusted according to improvements in physical fitness. The velocity of execution of resistance exercises during the concentric phase of the movement, the impact, height of jumps and amplitude of movements of weight bearing exercises, and the difficulty level of balance training will be also progressively increased.

Starting at month 2, a weighted vest will be worn during each session (while performing aerobic training and weight bearing exercises), and also outside the sessions (for at least 1 hour and during 3 10-repetition series of step-up and sit-to-stand in three non-training days every week). Patients will be asked to record in a daily diary the time spent wearing the weighted vest outside the sessions. Weight of vests will be 2% of body weight and will be increased by 2% every 6 months (i.e., up to 8%).

**Standard care**

All patients will be subjected to a treatment regimen aimed at achieving glycaemic, lipid, BP and body weight targets, as established by current guidelines and including nutritional therapy and glucose-, lipid- and BP-lowering agents as needed.[46] Vitamin D will be supplemented to maintain levels higher than 30 ng/ml.

At intermediates routine diabetes visits, drugs will be adjusted to attain target levels, following a pre-specified algorithm, and changes will be recorded into the SWEET-BONE database.

**Outcomes**

The primary endpoint is baseline to end-of-study change in TBS, based on previous reports showing that it is consistently lower in T2D versus non-diabetic individuals [15-20].

Secondary endpoints include (a) other potential measures of bone quality, as assessed by QUS and pQCT; (b) bone mass (BMD); (c) markers of bone turnover; (d) body composition; (e) muscle strength, mass, and power; (f) balance and gait; (g) number of falls; and (h) asymptomatic and symptomatic fractures. Falls and fractures will be evaluated over 7 years (i.e., including the 5-year post-trial follow-up).

PA level, other components of physical fitness, i.e., cardio-respiratory fitness and flexibility, MS symptoms, modifiable cardiovascular risk factors, medications, and coronary heart disease (CHD) and stroke 10-year risk scores will be also evaluated.

The assessors of outcome measures will be blinded to group assignment.

**Measurements**

***Bone mass and quality.*** Bone mass will be assessed by DXA scans of the lumbar spine (L1 to L4) and total femur using Hologic QDR 4500 W 2000 (Hologic, Bedford, MA, USA). Areal BMD (aBMD, g/cm$^2$) in the lumbar spine and femoral neck will be recorded and the corresponding T scores and Z scores will be obtained. Composite indices of femoral neck strength will be also computed from the femoral neck axis length (FNAL) and width (FNW), femoral neck aBMD, and body size, i.e. compression strength index (CSI), bending strength index (BSI), and impact strength index (CSI).[50] TBS will be then measured using the Hologic TBS Insight software (Hologic). Calcaneal QUS measurements will be performed using the Sahara® Clinical Bone Sonometer (Technologic, Turin, Italy). Broadband ultrasound attenuation (BUA; dB/MHz) and speed of sound (SOS; m/s) will be measured, and the quantitative ultrasound index (QUI) will be then calculated. BMD will be also estimated from QUS measurements (eBMD, g/cm$^2$). Bone density and macroarchitecture will be evaluated using an XCT-2000 pQCT scanner (Norland Stratec, Stratec, Pforzheim, Germany).[51] Slices (2.5 mm) will be obtained at the 4%, 14%, 38%, and 66% sites of the left tibia and at the 4% site of the nondominant radius. At the metaphyses (4% site) of tibia and radius, total vBMD (Tot-vBMD) and Trabecular (Trab)-vBMD (mg/cm$^3$) will be measured. At the 14% site of the tibia, cortical bone area (Cort-A, mm$^2$) and cortical bone mineral content (Cort-BMC, mg/cm), two markers of resistance to compressive and tensile loads, will be measured, and the section modulus will be calculated from the antero-posterior, latero-lateral, and polar moments of inertia (Ix, Iy, and Ip, respectively) and used to obtain the stress-strain index (SSI, mm$^3$), a surrogate measure of resistance to bending (xSSI and ySSI) and torsional (pSSI) loads. At the 38% site of the tibia, cortical vBMD (Cort- vBMD, mg/cm$^3$) and total cross-sectional area (Tot-CSA, mm$^2$) will measured, together with calculation of cortical thickness (CT, mm) and circularity index (CI), a proxy of tibial geometrical load adaptation.

***Markers of bone turnover.*** Serum calcium and phosphorus, 25OH Vitamin D, and parathyroid hormone will be measured, together with the following markers of bone turnover: total and bone-specific alkaline phosphatase, osteocalcin, and procollagen I intact N-terminal, for bone formation, and C-terminal telopeptide of type I collagen, tartrate-resistant acid phosphatase 5b, sclerostin, and Dickkopf-1, for bone resorption. These measurements will be centralized at the Laboratory of Clinical Chemistry of Sant'Andrea University Hospital, using the methods reported in Table 2.

***Body composition.*** Total body DXA will be used to evaluate body composition, with measurement of total body lean mass and total body fat mass.

***Muscle strength.*** Isometric muscle strength will be also assessed by means of a strain gauge tensiometer (Digimax, Mechatronic GmbH, Germany), as previously reported.[52] Maximal voluntary contractions are performed at the shoulder press (Technogym, Gambettola, Italy) along the sagittal plan, with a 45° and 90° angle at the elbow and between the upper arm and the trunk, respectively, for the upper body, and at the leg extension machine (Technogym), with a 90° angle at the knee and the hip, for the lower body. Values will be expressed in Nm for two arms.

***Muscle cross-sectional area.*** The cross-sectional areas of muscles of the leg will be measured by pQCT at the 66% site of the tibia at the end of bone assessments.[53]

***Physical fitness.*** Physical fitness will be evaluated at baseline, end-of-study and, in the EXE group, also at month 6, 12, and 18, in order to adjust training loads. Cardio-respiratory fitness, muscle fitness, and flexibility will be assessed by a sub-maximal evaluation of oxygen consumption at 80% of the maximal heart rate to predict maximal oxygen consumption ($VO_{2max}$), a maximal repetition (or 5-8 RM) to predict one-repetition maximum (1-RM), and a standard bending test, respectively, as previously reported.[49, 54]

***Balance, gait and power.*** A "Short Battery Performance Test" will be performed for the assessment of balance (side-by-side stand, semi-tandem stand and tandem stand), gait (gait speed test) and power (chair stand test).[55]

***Number of falls.*** Falls will be recorded using the 17-item History of Falls questionnaire (see Appendix B1).[56]

***Symptomatic and asymptomatic fractures.*** Patients will be interviewed to record symptomatic fractures, which will be adjudicated based on clinical and radiographic records. Asymptomatic fractures will be identified by vertebral morphometry.

***PA level.*** The level of PA will be evaluated throughout the study by asking patients to fill in the PASE questionnaire (see Appendix B2), a validated instrument for the measurement of PA level in individuals aged ≥65 years.[57] The amount of supervised exercise in the EXE group will be measured as previously reported.[49, 54]

***MS symptoms.*** MS symptoms will be evaluated by a 50-item self-report questionnaire (see Appendix B3).[58]

***Cardiovascular risk factors and scores.*** The BMI will be calculated from body weight and height, while waist circumference will be taken at the umbilicus and BP will be recorded with a sphygmomanometer after a five-minute rest with the patient seated. Blood and urine samples will be taken for measuring the biochemical parameters reported in Table 3 at the Laboratory of Clinical Chemistry of Sant'Andrea University Hospital. Global and fatal CHD and stroke 10-year risk scores will be calculated using the United Kingdom Prospective Diabetes Study (UKPDS) risk engine.[59] Cardiovascular risk factors and scores will be assessed at baseline, year 1, end-of-study, and, eventually, also at month 6 and 18, in order to adjust treatment.

**Adverse events**

Adverse events will be reported at intermediate visits and, for EXE participants, also at supervised sessions, by completing a standard form.

The risk of injuries and other adverse events during the training sessions will be covered by an insurance (N. 390-01583709-14010, HDI-Gerling Industrie Versicherung AG, Leipzig, Deutschland).

**Data collection, storage and security**

Data collected into the SWEET-BONE database will be saved to a password-protected server in the Metabolic Fitness Association and accessed only by members of the research team.

Once uploaded to the server, data will be securely deleted from the recording devices. Patient questionnaire data will be made anonymous and stored in locked filing cabinets.

**Statistical analysis**

Sample size calculation was based on our pilot study showing that TBS was 1.225±0.085 (SD) in T2D individuals. To detect a between-group difference of 0.045 in TBS (i.e., effect size=0.50) with statistical power of 90% (α=0.05) by two-sided two-sample equal-variance t-test, 86 patients per arm are needed. A sample of 200 patients allows to tolerate an 14% dropout rate.

The $\chi^2$ or, where appropriate, the Fisher's exact test, for categorical variables, and the Student's t test or the corresponding nonparametric Mann-Whitney test for continuous variables will be utilized to compare patients' characteristics at baseline. The intention-to-treat analysis will be applied to all randomized patients. The superiority of the intervention on the primary and secondary endpoints will be assessed by mixed models for repeated measures. Pre-specified subgroup analyses will be conducted by gender, age (65-70 versus 71-75), and type of diabetes treatment (non-insulin versus insulin).

To account for change in medication throughout the study period, which might affect bone parameters, we will perform both multiple regression and sensitivity analyses. In the regression models, the dependent variable will be represented by baseline to end-of-study changes. Treatment at baseline and treatment initiation during the study will be included in the model as dichotomous variables (yes versus no), whereas drug dosage will be not taken into consideration. Sensitivity analysis will be conducted by comparing study arms after exclusion of patients who modified treatment.

Repeated measures models with an autoregressive correlation type matrix make an assumption of missing at random and account for both missingness at random and potential correlation within participants, as they

allow evaluating all individuals, including those with incomplete data.[60] Finally, to guarantee replicability and avoid outcome selective reporting, a fully specified statistical analysis plan will be written before unmasking.

Statistical analyses will be performed by at the CORESEARCH using SAS software release 9.3 (Cary, NC, USA) and the statistical significance level will be set at $\alpha < 0.05$ (2-tailed). Because of the potential for type 1 error due to multiple comparisons, findings for analyses of secondary endpoints should be interpreted as exploratory.

**Patient and public involvement**

Patients or public will not be involved in the study, except for the burden of the intervention, which will be assessed by patients themselves and reported to the exercise specialist at each session, in order to identify the appropriate training modalities to minimize the risk of injury or adverse events.

## Ethics and Dissemination

The research protocol (version #3, February 28, 2013), which follows the SPIRIT guideline (see Appendix C), complies with the Declaration of Helsinki. It has been approved by the Ethics Committee of Sant'Andrea University Hospital on 21 March, 2013 (Prot. n. 2583/2013) and has been registered with ClinicalTrials.gov on 20 April. 2014 (NCT02421393; URL https://clinicaltrials.gov/ct2/show/NCT02421393) (see Appendix D). Important protocol amendments (e.g., changes to eligibility criteria, outcomes, analyses) will be communicated to relevant parties, i.e., investigators, trial participants, trial registry, and the Ethics Committee.

All participants will provide written informed consent (see Appendix E) following verbal and written explanation of the study protocol and the opportunity to ask questions. Participants will not be provided with an honorarium and will be free to withdraw from the trial at any time without prejudice to future treatment.

To the best of our knowledge, the SWEET-BONE is the first study investigating whether a specifically designed exercise training program is effective in improving bone quality and strength in patients with T2D, thus potentially reducing the increased fracture risk characterizing these individuals despite preserved bone mass. The beneficial effects on bone quality would be additional to those on muscle strength and mass and risk of fall, which may reduce per se the risk of fracture. However, generalizability and implementation in clinical practice of this approach will require further investigation and validation in different cohorts or contexts.

Results will be presented at scientific meetings and published in peer-reviewed journals. All publications and presentations related to the study will be authorized and reviewed by the study investigators. The ICMJE Recommendations will be adopted for authorship [61].

After publication of results, public access to the full protocol, participant-level dataset, and statistical code will be eventually granted upon request.

## References


1. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. *Osteoporos Int.* 2007;18:427–444.
2. Janghorbani M, Van Dam RM, Willett WC, et al. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *Am J Epidemiol.* 2007;166:495–505.
3. Schwartz AV, Sellmeyer DE, Ensrud KE, et al. Older women with diabetes have an increased risk of fracture: a prospective study. *J Clin Endocrinol Metab.* 2001;86:32–38.
4. Strotmeyer ES, Cauley JA, Schwartz AV, et al. Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: The Health, Aging, and Body Composition Study. *Arch Intern Med.* 2005;165:1612–1617.
5. Napoli N, Strotmeyer ES, Ensrud KE, et al. Fracture risk in diabetic elderly men: the MrOS study. *Diabetologia.* 2014;57:2057–2065.
6. Bonds DE, Larson JC, Schwartz AV, et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. *J Clin Endocrinol Metab.* 2006;91:3404–3410.
7. Schwartz AV, Hillier TA, Sellmeyer DE, et al. Older women with diabetes have a higher risk of falls: a prospective study. *Diabetes Care.* 2002;25:1749–1754.
8. Schwartz AV, Vittinghoff E, Bauer DC, et al. Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. *JAMA.* 2011;305:2184–2192.
9. Fonseca H, Moreira-Gonçalves D, Coriolano HJ, et al. Bone quality: the determinants of bone strength and fragility. *Sports Med.* 2014;44:37–53.
10. Farr JN, Khosla S. Determinants of bone strength and quality in diabetes mellitus in humans. *Bone.* 2016;82:28–34.
11. Rubin MR, Patsch JM. Assessment of bone turnover and bone quality in type 2 diabetic bone disease: current concepts and future directions. *Bone Res.* 2016;4:16001.

12. Jiang N, Xia W. Assessment of bone quality in patients with diabetes mellitus. *Osteoporos Int.* 2018;29:1721–1736.

13. Bousson V, Bergot C, Sutter B, et al. Trabecular bone score (TBS): available knowledge, clinical relevance, and future prospects. *Osteoporos Int.* 2012;23:1489–1501.

14. Zhukouskaya VV, Eller-Vainicher C, Gaudio A, et al. The utility of lumbar spine trabecular bone score and femoral neck bone mineral density for identifying asymptomatic vertebral fractures in well-compensated type 2 diabetic patients. *Osteoporos Int.* 2016;27:49–56.

15. Leslie WD, Aubry-Rozier B, Lamy O, et al. TBS (trabecular bone score) and diabetes-related fracture risk. *J Clin Endocrinol Metab.* 2013;98:602–609.

16. Dhaliwal R, Cibula D, Ghosh C, et al. Bone quality assessment in type 2 diabetes mellitus. *Osteoporos Int.* 2014;25:1969–1973.

17. Kim JH, Choi HJ, Ku EJ, et al. Trabecular bone score as an indicator for skeletal deterioration in diabetes. *J Clin Endocrinol Metab.* 2015;100:475–482.

18. Choi YJ, Ock SY, Chung YS. Trabecular Bone Score (TBS) and TBS-adjusted fracture risk assessment tool are potential supplementary tools for the discrimination of morphometric vertebral fractures in postmenopausal women with type 2 diabetes. *J Clin Densitom*. 2016;19:507–514.

19. Holloway KL, De Abreu LLF, Hans D, et al. Trabecular bone score in men and women with impaired fasting glucose and diabetes. *Calcif Tissue Int.* 2018;102:32–40.

20. Rianon N, Ambrose CG, Buni M, et al. Trabecular bone score is a valuable addition to bone mineral density for bone quality assessment in older Mexican American women with type 2 diabetes. *J Clin Densitom.* 2018;21:355–359.

21. Glüer CC. Quantitative ultrasound techniques for the assessment of osteoporosis: expert agreement on current status. The International Quantitative Ultrasound Consensus Group. *J Bone Miner Res.* 1997;12:1280–1288.

22. Patel S, Hyer S, Tweed K, et al. Risk factors for fractures and falls in older women with type 2 diabetes mellitus. *Calcif Tissue Int.* 2008;82:87–91.

23. Padilla F, Jenson F, Bousson V, et al. Relationships of trabecular bone structure with quantitative ultrasound parameters: In vitro study on human proximal femur using transmission and backscatter measurements. *Bone.* 2008;42:1193–1202.

24. Bouxsein ML, Radloff SE. Quantitative ultrasound of the calcaneus reflects the mechanical properties of calcaneal trabecular bone. *J Bone Miner Res.* 1997;12:839–846.

25. Patel S, Hyer S, Tweed K, et al. Risk factors for fractures and falls in older women with type 2 diabetes mellitus. *Calcif Tissue Int.* 2008;82:87–91.

26. Yamaguchi T, Yamamoto M, Kanazawa I, et al. Quantitative ultrasound and vertebral fractures in patients with type 2 diabetes. *J Bone Miner Metab.* 2011;29:626–632.

27. Tao B, Liu JM, Zhao HY, et al. Differences between measurements of bone mineral densities by quantitative ultrasound and dual-energy X-ray absorptiometry in type 2 diabetic postmenopausal women. *J Clin Endocrinol Metab.* 2008;93:1670–1675.

28. Bulló M, Garcia-Aloy M, Basora J, et al. Bone quantitative ultrasound measurements in relation to the metabolic syndrome and type 2 diabetes mellitus in a cohort of elderly subjects at high risk of cardiovascular disease from the PREDIMED study. *J Nutr Health Aging*. 2011;15:939–944.

29. Patsch JM, Burghardt AJ, Yap SP, et al. Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. *J Bone Miner Res.* 2013;28:313–324.

30. Samelson EJ, Demissie S, Cupples LA, et al. Diabetes and deficits in cortical bone density, microarchitecture, and bone size: Framingham HR-pQCT Study. *J Bone Miner Res.* 2018;33:54–62.

31. Burghardt AJ, Issever AS, Schwartz AV, et al. High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab.* 2010;95:5045–5055.

32. Farr JN, Drake MT, Amin S, et al. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. *J Bone Miner Res.* 2014;29:787–795.

33. Paccou J, Ward KA, Jameson KA, et al. Bone microarchitecture in men and women with diabetes: the importance of cortical porosity. *Calcif Tissue Int.* 2016;98:465–473.

34. Nilsson A, Sundh D, Johansson L, et al. Type 2 diabetes mellitus is associated with better bone microarchitecture but lower bone material strength and poorer physical function in elderly women: a population-based study. *J Bone Miner Res.* 2017;32:1062–1071.

35. Arnett TR. Osteocytes: regulating the mineral reserves? *J Bone Miner Res.* 2013;28:2433–2435.

36. Kerschnitzki M, Kollmannsberger P, Burghammer M, et al. Architecture of the osteocyte network correlates with bone material quality. *J Bone Miner Res.* 2013;28:1837–1845.

37. Sinaki M, Pfeifer M, Preisinger E, et al. The role of exercise in the treatment of osteoporosis. *Curr Osteoporos Rep.* 2010;8:138–144.

38. Howe TE, Shea B, Dawson LJ, et al. Exercise for preventing and treating osteoporosis in postmenopausal women. *Cochrane Database Syst Rev.* 2011;(7):CD000333.

39. Karlsson M. Does exercise reduce the burden of fractures? A review. *Acta Orthop Scand.* 2002;73:691–705.

40. Sinaki M, Itoi E, Wahner HW, et al. Stronger back muscles reduce the incidence of vertebral fractures: a prospective 10 year follow-up of postmenopausal women. *Bone.* 2002;30:836–841.

41. Villareal DT, Chode S, Parimi N, et al. Weight loss, exercise, or both and physical function in obese older adults. *N Engl J Med.* 2011;364:1218–1229.

42. Shah K, Armamento-Villareal R, Parimi N, et al. Exercise training in obese older adults prevents increase in bone turnover and attenuates decrease in hip bone mineral density induced by weight loss despite decline in bone-active hormones. *J Bone Miner Res.* 2011;26:2851–2859

43. Villareal DT, Aguirre L, Gurney AB, et al. Aerobic or resistance exercise, or both, in dieting obese older adults. *N Engl J Med.* 2017;376:1943–1955.

44. Gillespie LD, Gillespie WJ, Robertson MC, et al. Interventions for preventing falls in elderly people. *Cochrane Database Syst Rev.* 2003;(4):CD000340.

45. Orlando G, Balducci S, Bazzucchi I, et al. Neuromuscular dysfunction in type 2 diabetes: underlying mechanisms and effect of resistance training. *Diabetes Metab Res Rev.* 2016;32:40–50.

46. American Diabetes Association. Standards of Medical Care in Diabetes—2018. *Diabetes Care.* 2018;41(Suppl. 1):S1–S159.

47. Inoue M, Iso H, Yamamoto S, et al. Daily total physical activity level and premature death in men and women: results from a large-scale population-based cohort study in Japan (JPHC study). *Ann Epidemiol.* 2008;18:522–530.

48. Sedentary Behaviour Research Network. Letter to the Editor: Standardized use of the terms "sedentary" and "sedentary behaviours". *Appl Physiol Nutr Metab.* 2012;37:540–542.

49. Balducci S; Zanuso S; Nicolucci A; et al. Effect of an intensive exercise intervention strategy on modifiable cardiovascular risk factors in type 2 diabetic subjects. A Randomized Controlled Trial: The Italian Diabetes and Exercise Study (IDES). *Arch Intern Med.* 2010;170:1794–1803.

50. Ishii S, Cauley JA, Crandall CJ, et al. Diabetes and femoral neck strength: findings from the Hip Strength Across the Menopausal Transition Study. *J Clin Endocrinol Metab.* 2012;97:190–197.

51. Russo CR, Lauretani F, Seeman E, et al. Structural adaptations to bone loss in aging men and women. *Bone.* 2006;38:112–118.

52. Balducci S, Sacchetti M, Orlando G, et al. Correlates of muscle strength in diabetes. The Study on the Assessment of Determinants of Muscle and Bone Strength Abnormalities in Diabetes (SAMBA). *Nutr Metab Cardiovasc Dis.* 2014;24:18–26.

53. Erlandson MC, Lorbergs AL, Mathur S, et al. Muscle analysis using pQCT, DXA and MRI. *Eur J Radiol.* 2016;85:1505–1511.

54. Balducci S, Zanuso S, Massarini M, et al. The Italian Diabetes and Exercise Study (IDES): Design and methods for a prospective Italian multicentre trial of intensive lifestyle intervention in people with type 2 diabetic and the metabolic Syndrome. *Nutr Metab Cardiovasc Dis.* 2008;18:585–595.

55. Guralnik JM, Ferrucci L, Simonsick EM, et al. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med.* 1995;332:556–561.

56. Talbot LA, Musiol RJ, Witham EK, et al. Falls in young, middle-aged and older community dwelling adults: perceived cause, environmental factors and injury. *BMC Public Health.* 2005;18;5:86.

57. Washburn RA, Smith KW, Jette AM, et al. The physical activity scale for the elderly (PASE): Development and evaluation. *J Clin Epidemiol.* 1993;46:153–162.

58. Balducci S, Vulpiani MC, Pugliese L, et al. Effect of supervised exercise training on musculoskeletal symptoms and function in patients with type 2 diabetes: the Italian Diabetes Exercise Study (IDES). *Acta Diabetol.* 2014;51:647–654.

59. Stevens RJ, Kothari V, Adler AI, et al. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). *Clin Sci (Lond).* 2001;101:671–679.

60. Singer JD, Willett JB. Applied longitudinal data analysis: modeling change and event occurrence. New York, NY, USA: Oxford University Press 2003.

61. International Committee of Medical Journal Editors. Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. 2017, http://www.icmje.org/icmje-recommendations.pdf (accessed 18 October, 2018).

**Authors' contributions:** SB, FGC and GP conceived and designed the study. All the other authors made substantial contributions to specific parts of the protocol: recruitment and follow-up program (MV and LB), training program (MS, GO, GR, and SZ), imaging procedures (GA, LP, and AL), QUS and pQCT protocols (CRR, JH, and VDE), biochemical testing panel (PC), and statistical analysis plan (AN). GP drafted the manuscript; SB, FGC, MS, CRR, GA, JH, GO, GR, VDE, PC, LP, AL, MV, LB, SZ, and AN revised the manuscript critically for important intellectual content. All authors have given their final approval of the manuscript to be published.

**Funding statement:** This work is supported by the Metabolic Fitness Association, Monterotondo, Rome, Italy and, for the pilot study, by a grant from the Italian Ministry of Health (RF-2010-2310812) to GP. The funding sources have no role in design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the report for publication.

**Declaration of interest:** The authors declare no competing interests.

## Figure Legends

**Figure 1.** Study flow chart. LTPA = leisure-time physical activity; PASE = Physical Activity Scale for the Elderly; MS = musculoskeletal; DXA = dual-energy X-ray absorptiometry; QUS = quantitative ultrasound; pQCT = peripheral quantitative computed tomography.

**Figure 2.** Sequence of exercises during each supervised exercise training session. * Intensity of aerobic exercise will be adjusted according to improvements in predicted $VO_{2max}$, as recorded every 6 months. † Intensity of resistance exercise will be adjusted according to improvements in 1-RM, as recorded every 6 months; new resistance exercises will be introduced every 12 weeks to maintain patient's adherence, and the velocity of execution during the concentric phase of the movement will be progressively increased to enhance muscle power. ‡ Height of jumps and amplitude of movements of weight bearing exercise will be also progressively increased. § Difficulty level of balance training will be gradually increased by performing the exercises with closed eyes, reducing the support area, changing visual fixation (e.g., head rotations), varying the centre of mass (e.g., limb raising), or adding a manual or cognitive task. $VO_{2max}$ = maximal oxygen consumption; 1-RM = one-repetition maximum.

**Table 1.** Exclusion criteria.

- Unable or unwilling to give informed consent or communicate with local study staff
- Current diagnosis of psychiatric disorder or hospitalization for depression in the past six months
- Self-reported alcohol or substance abuse within the past twelve months
- Self-reported inability to walk two blocks
- Musculoskeletal disorders or deformities that may interfere with participation in the intervention
- History of central nervous dysfunction such as hemiparesis, myelopathies, cerebral ataxia
- Clinical evidence of vestibular dysfunction
- Postural hypotension defined as a fall in BP when changing position of >20 mmHg (systole) or >10 mmHg (diastole)
- Cancer requiring treatment in the past five years, except for cancers that have clearly been cured or in the opinion of the investigator carry an excellent prognosis (e.g., stage 1 cervical cancer)
- Chronic obstructive pulmonary disease
- End-stage liver disease
- Chronic diabetic complications:
  - recent major acute cardiovascular event, including heart attack, stroke/transient ischemic attack(s), revascularization procedure, or participation in a cardiac rehabilitation program within the past three months
  - pre-proliferative and proliferative retinopathy
  - macroalbuminuria and/or eGFR < 45 ml/min/1.73 $m^2$
  - severe motor and sensory neuropathy
  - diabetic foot with history of ulcer
- Cardiovascular disease at cardiologic examination:
  - history of cardiac arrest
  - history of pulmonary embolism in the past six months
  - unstable angina pectoris or angina pectoris at rest
  - resting HR <45 beats/min or >100 beats/min
  - complex ventricular arrhythmia at rest or with exercise
  - uncontrolled atrial fibrillation (HR $\geq$100 beats/min)
  - NYHA Class III or IV congestive heart failure
  - acute myocarditis, pericarditis or hypertrophic myocardiopathy
  - left bundle branch block or cardiac pacemaker

- ECG treadmill test suggestive of myocardial ischemia

- Poor glycaemic and blood pressure (BP) control
  - haemoglobin (Hb) $A_{1c}$ >9.0%
  - BP >150/90 mmHg
- Bone abnormalities
  - vitamin D <10 ng/ml
  - treatment with anti-fracturative agents, estrogens, aromatase inhibitors, testosterone, corticosteroids and/or glitazones
  - previous documented non-traumatic fractures
  - SDI >5 (and >2 in a single vertebra)
  - T score ≤-2.5 at spine/hip at DXA
- Conditions not specifically mentioned above at the discretion of the clinical site

---

BP = blood pressure; eGFR = estimated glomerular filtration rate; HR = heart rate; NYHA = New York Heart Association; ECG = electrocardiogram; SDI = total spine deformity index; DXA = dual-energy X-ray absorptiometry. Participants with $HbA_{1c}$ or BP above the indicated threshold will be receive appropriate treatment and will be re-evaluated after 3 months. Patients with vitamin D levels < 10 ng/dl will be treated with cholecalciferol 25.000 IU/week for 6 weeks and will be re-evaluated 2 weeks after the last dose.

**Table 2.** Methods for measurements of markers of bone turnover.

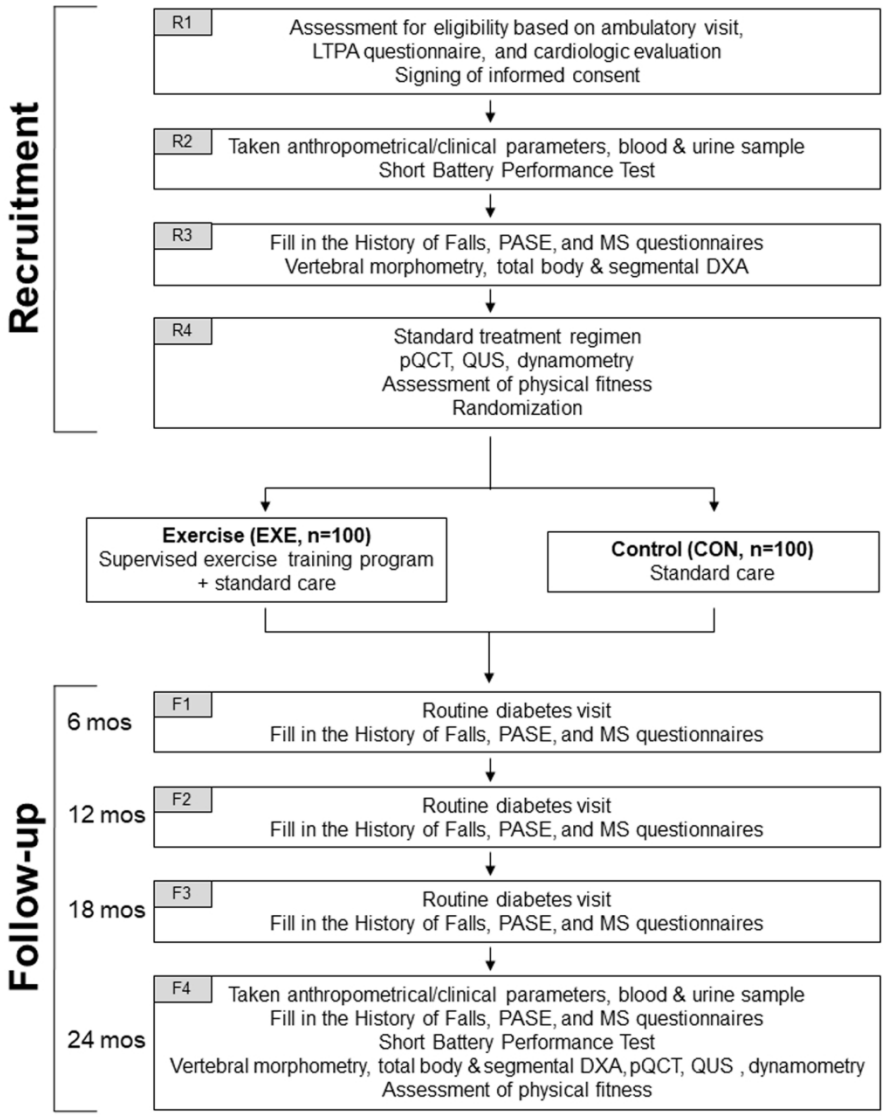
| Analyte | Method | Manifacturer |
|---|---|---|
| **Ca** | Colorimetric spectrophotometric | Architect, Abbot Diagnostics, Lake Forest, IL, USA |
| **P** | Colorimetric spectrophotometric | Architect, Abbot Diagnostics, Lake Forest, IL, USA |
| **25OH Vitamin D** | Competitive ECLIA | Liaison, DiaSorin SpA, Saluggia, Italy |
| **PTH** | ECLIA | Liaison, DiaSorin SpA, Saluggia, Italy |
| **Total ALP** | Colorimetric spectrophotometric | Architect, Abbot Diagnostics, Lake Forest, IL, USA |
| **Bone-specific ALP** | ECLIA | Liaison, DiaSorin SpA, Saluggia, Italy |
| **Osteocalcin** | ELISA | RayBiotech, Norcross, GA, USA |
| **PINP** | ELISA | RayBiotech, Norcross, GA, USA |
| **CTX-1** | ELISA | RayBiotech, Norcross, GA, USA |
| **TRAcP 5b** | ELISA | RayBiotech, Norcross, GA, USA |
| **Sclerostin** | ELISA | RayBiotech, Norcross, GA, USA |
| **DKK-1** | ELISA | RayBiotech, Norcross, GA, USA |

Ca = calcium; P = phosphorus; PTH = parathyroid hormone; ALP = alkaline phosphatase; PINP = procollagen I intact N-terminal; CTX-1 = C-terminal telopeptide of type I collagen; TRAcP 5b = tartrate-resistant acid phosphatase 5b; DKK-1= Dickkopf-1; ECLIA = chemiluminescent immunoassay; ELISA; enzyme-linked immunosorbent assay.

**Table 3.** Methods for measurements of cardiovascular risk factors.

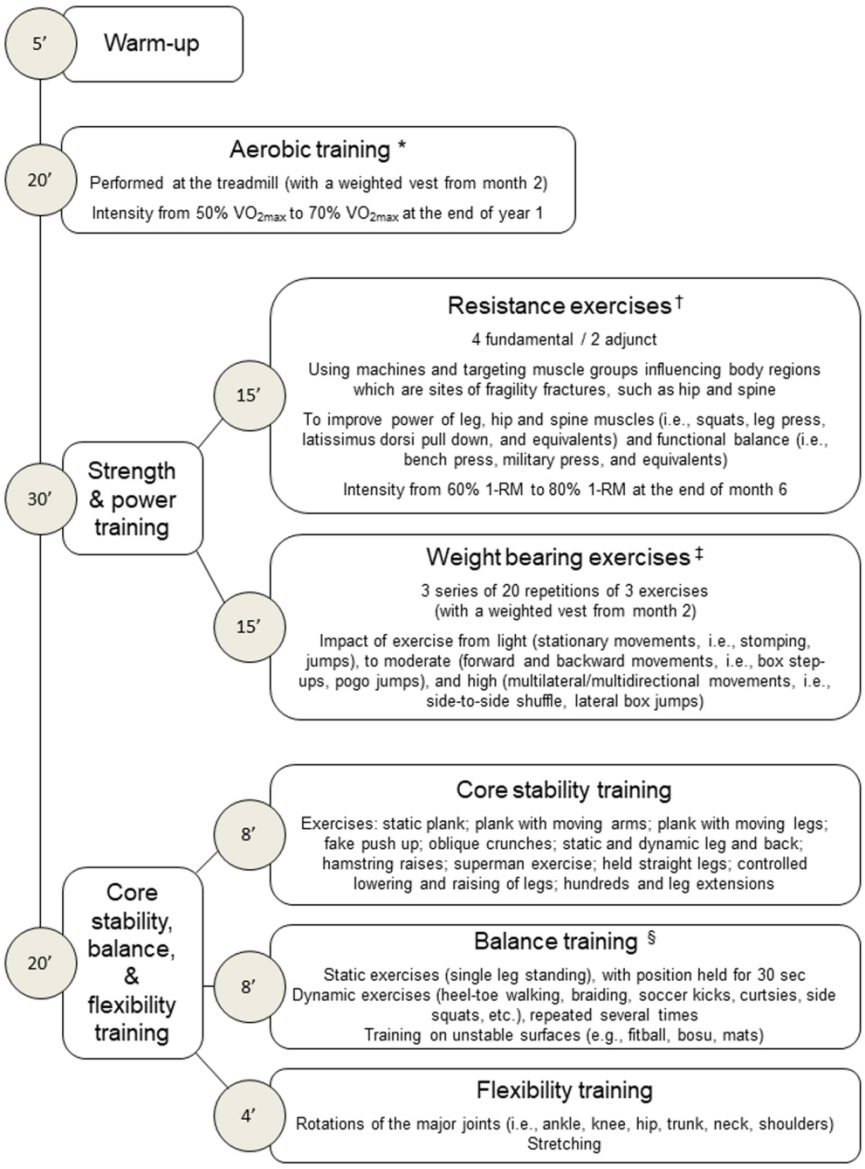
| Analyte | Method | Manifacturer |
|---|---|---|
| **$HbA_{1c}$** | HPLC (Adams TMA1C HA-8160) | Menarini Diagnostics, Florence, Italy |
| **FPG** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Triglycerides** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Total cholesterol** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **HDL cholesterol** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **hs-CRP** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Blood count** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Uric acid** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Serum creatinine** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Urinary albumin** | mAlb VITROS | Ortho Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Urinary creatinine** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |

$HbA_{1c}$ = haemoglobin $A_{1c}$; HPLC = high-performance liquid chromatography; FPG = fasting plasma glucose; hs-CRP= high sensitivity-C-reactive protein. LDL cholesterol will be calculated using the Friedewald formula (https://www.mdcalc.com/ldl-calculated), whereas glomerular filtration rate (GFR) will be estimated from serum creatinine by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (http://www.qxmd.com/calculate-online/nephrology/ckd-epi-egfr).




Study flow chart. LTPA = leisure-time physical activity; PASE = Physical Activity Scale for the Elderly; MS = musculoskeletal; DXA = dual-energy X-ray absorptiometry; QUS = quantitative ultrasound; pQCT = peripheral quantitative computed tomography.

142x189mm (300 x 300 DPI)




Sequence of exercises during each supervised exercise training session. * Intensity of aerobic exercise will be adjusted according to improvements in predicted VO2max, as recorded every 6 months. † Intensity of resistance exercise will be adjusted according to improvements in 1-RM, as recorded every 6 months; new resistance exercises will be introduced every 12 weeks to maintain patient's adherence, and the velocity of execution during the concentric phase of the movement will be progressively increased to enhance muscle power. ‡ Height of jumps and amplitude of movements of weight bearing exercise will be also progressively increased. § Difficulty level of balance training will be gradually increased by performing the exercises with closed eyes, reducing the support area, changing visual fixation (e.g., head rotations), varying the centre of mass (e.g., limb raising), or adding a manual or cognitive task. $VO_{2max}$ = maximal oxygen consumption; 1-RM = one-repetition maximum.

142x189mm (300 x 300 DPI)

## Appendix A: List of participants

**Recruitment and follow-up:** Diabetes Unit, Sant'Andrea Hospital, Rome, Italy: Giuseppe Pugliese, Stefano Balducci, Martina Vitale, Tiziana Cirrito, Lucilla Bollanti, Francesco G. Conti.

**Supervised exercise training:** Metabolic Fitness Association, Monterotondo, Rome, Italy: Stefano Balducci, Gianluca Balducci, Enza Spinelli.

**DXA and vertebral morphometry evaluation:** Radiology Unit, Sant'Andrea University Hospital, Rome, Italy Giuseppe Argento, Luca Pugliese, Andrea Laghi.

**QUS and pQCT evaluation:** Metagym Fitness Centre, Florence, Italy: Cosimo R. Russo; Diabetes Unit, Sant'Andrea Hospital, Rome, Italy: Jonida Haxhi, Valeria D'Errico.

**Physical Fitness evaluation:** Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy: Massimo Sacchetti, Giorgio Orlando, Olimpia Andreani; Diabetes Unit, Sant'Andrea Hospital, Rome, Italy: Gianvito Rapisarda, Eugenio Santacroce.

**Questionnaire evaluation:** Centre for Applied Biological & Exercise Sciences, Faculty of Health & Life Sciences, Coventry University, Coventry, UK: Silvano Zanuso.

**Laboratory testing:** Laboratory of Clinical Chemistry, Sant'Andrea Hospital, Rome, Italy: Patrizia Cardelli, Gerardo Salerno, Stefano Cavallo.

**Statistical Analysis:** Centre for Outcomes Research and Clinical Epidemiology (CORE), Pescara, Italy: Antonio Nicolucci, Giuseppe Lucisano.

**Steering Committee:** Giuseppe Pugliese, Stefano Balducci, Francesco G. Conti, Massimo Sacchetti, Cosimo R, Russo, Giuseppe Argento, Silvano Zanuso, Patrizia Cardelli, Antonio Nicolucci.

## Appendix B: Questionnaires

**B1: History of Falls questionnaire**

**B2: Physical Activity Scale for the Elderly (PASE) questionnaire**

**B3: Self-report questionnaire for musculoskeletal (MS) symptoms**

## B1: History of Falls questionnaire

| | |
|---|---|
| **A. Activities prior to falling** | |
| 1. Ambulation | |
| 2. Transferring | |
| 3. Running | |
| 4. Sports | |
| 5. Stairs/curb | |
| 6. Other | |
| **B. Perceived causes (accident/environmental-related)** | |
| 1. Collapse episode | |
| 2. Dizziness/vertigo | |
| 3. Balance/gait impairment | |
| 4. Other | |
| **C. Perceived causes (environmental factors)** | |
| 1. Wet surface | |
| 2. Uneven surface/steps | |
| 3. Objects on surface/rugs | |
| 4. External forces | |
| 5. Icy surfaces | |
| 6. Other | |
| **D. Injuries sustained from fall** | |
| 1. Fractures | |
| 2. Treated injury | |
| 3. Untreated injury | |
| 4. No injury | |

## B2: Physical Activity Scale for the Elderly (PASE) questionnaire

**Q1.** Over the past 7 days, how often did you participate in sitting activities such as reading, watching TV, or doing handcrafts?

**Q1b.** On average, how many hours per day did you engage in these sitting activities?

**Q2.** Over the past 7 days, how often did you take a walk outside your home or yard for any reason? For example, for fun or exercise, walking to work, walking the dog, walking in a mall, etc?

**Q2a.** On average, how many hours per day did you spend walking?

**Q3.** Over the past 7 days, how often did you engage in light sport or recreational activities such as bowling, golf with a cart, shuffleboard, fishing from a boat or pier or other similar activities?

**Q3b.** On average, how many hours per day did you engage in these light sport or recreational activities?

**Q4.** Over the past 7 days, how often did you engage in moderate sport or recreational activities such as doubles tennis, ballroom dancing, hunting, ice skating, golf without a cart, softball or other similar activities?

**Q4b.** On average, how many hours per day did you engage in these moderate sport or recreational activities?

**Q5.** Over the past 7 days, how often did you engage in strenuous sport or recreational activities such as jogging, swimming, cycling, singles tennis, aerobic dance, skiing (downhill or cross country or other similar activities?

**Q5b.** On average, how many hours per day did you engage in these strenuous activities?

**Q6.** Over the past 7 days, how often did you do any exercises specifically to increase muscle strength or endurance, such as lifting weights or pushups, etc?

**Q6a.** On average, how many hours per day did you engage in exercises to increase muscle strength or endurance, such as lifting weights, pushups, or physical therapy with weights, etc.?

**Q7.** During the past 7 days, have you done any light housework, such as dusting, washing or drying dishes, or ironing?

**Q8.** During the past 7 days, have you done any heavy housework or chores such as vacuuming, scrubbing floors, washing windows, or carrying wood?

**Q9a.** During the past 7 days, did you engage in home repairs like painting, wallpapering, electrical work, etc.?

**Q9b.** During the past 7 days, did you engage in lawn work or yard care, including snow or leaf removal, chopping wood, etc?

**Q9c.** During the past 7 days, did you engage in outdoor gardening?

**Q9d.** During the past 7 days, did you engage in caring for another person such as a child, dependent spouse, or another adult?

**Q10.** During the past 7 days, did you work for pay or as a volunteer?

**Q10a.** How many hours per week did you work for pay and/or as a volunteer?

**Q10b.** Which of the following categories best describes the amount of physical activity required on your job and/or volunteer work?

Category 1 ("Mainly sitting with slight arm movements") includes examples such as: office worker, watchmaker, seated assembly line worker, bus driver, etc.

Category 2 ("Sitting or standing with some walking") includes examples such as: cashier, general office worker, light tool and machinery worker.

Category 3 ("Walking, with some handling of materials generally weighing less than 50 pounds") includes examples such as: mailman, waiter/waitress, construction worker, heavy tool and machinery worker.

Category 4 ("Walking and heavy manual work often requiring handling of materials weighing over 50 pounds") includes examples such as: lumberjack, stonemason, farm or general labourer].

## B3. Self-reported questionnaire for MS symptoms

| | |
|---|---|
| **SHOULDER** | |
| **1** | Do you have pain during rotation of the arm? |
| **2** | Are you awakened by pain during the night? |
| **3** | Do you have pain on reaching objects above the head? |
| **4** | Do you have pain on lifting objects? |
| **5** | Do you have pain or soreness upon awakening that passes later on during the day? |
| **6** | Have you taken anti-inflammation drugs or pain-killers? |
| **ARM** | |
| **7** | Do you feel that you have less strength? |
| **8** | Dou you feel that one arm is weaker than the other? |
| **9** | Do you have pain at the maximum extension of the forearm? |
| **ELBOW** | |
| **10** | Do you have pain on lifting an object? |
| **11** | Do you have pain on hitting against a rigid object? |
| **12** | Have you taken anti-inflammation drugs or pain-killers? |
| **WRIST** | |
| **13** | Do you have pain on lifting an object? |
| **14** | Do you have pain on hitting against a rigid object? |
| **15** | Have you taken anti-inflammation drugs or pain-killers? |
| **HAND:** Do you feel "pins and needles"? If so, in which finger? | |
| **16** | I |
| **17** | II |
| **18** | III |
| **19** | IV |
| **20** | V |
| **SPINE: THORACO-CERVICAL** | |
| **21** | Do you have pain/tenderness/ pins and needles on turning your head from side to side? |
| **22** | Do you often have pain or headache or heaviness of the head or neck? |
| **23** | Do you have pain between the shoulder blades? |
| **24** | Do you feel it necessary to move your head from side to side to get moving and feel? |
| **25** | Do you have episodes of painful sudden acute stiffness of the neck? |
| **26** | Have you taken anti-inflammation drugs or pain-killers? |
| **SPINE: LUMBO-SACRAL** | |
| **27** | Do you have pain on bending to tie your shoe laces? |
| **28** | Do you have any back-pain on turning left or right? |
| **29** | Do you have a feeling of heaviness in your back on standing for long hours? |
| **30** | Do you have bothersome feeling when sitting still? Do you have to get up? |
| **31** | Did you have one episode of sudden intense back pain that leaves you unable to move? |
| **32** | Have you taken anti-inflammation drugs or pain-killers? |

| | |
|---|---|
| **HIP** | |
| **33** | Do you have pain on crossing your legs? |
| **34** | Do you have any pain when opening your legs to the maximum? |
| **35** | Do you often have pain from your buttocks along the length of the leg down to your ankles? |
| **36** | Have you taken a single dose of anti-inflammatory drugs or pain-killers? |
| **KNEE** | |
| **37** | Do you have pain in the knee in the act of sitting down or getting up? |
| **38** | Do you have pain in your knee after having walked a lot? |
| **39** | Is your knee often swollen at the end of the day? |
| **40** | Do you have pain in the "good" knee? |
| **41** | Do you have pain or a bothersome feeling as you kneel down? |
| **42** | When lying in bed, do you feel the need to move your legs, ones or more than once? |
| **43** | Have you taken a single dose of anti-inflammatory drugs or pain-killers? |
| **FOOT** | |
| **44** | Do you often feel a sensation of pins and needles that runs down to one or more toes? |
| **45** | Do you have any difficulty in standing on your toes? |
| **46** | Do you have any pain in your foot after walking for a long time? |
| **47** | Do you have pain on taking the first step in the morning? |
| **48** | Do you have any difficulty or pain when putting on stiff orthopaedic shoes? |
| **49** | Do you have pain under the heel when walking a lot? |
| **50** | Have you taken a single dose of anti-inflammatory drugs or pain-killers? |

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

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

| Section/item | Item No | Description | Addressed on page number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 4, 17 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | Appendix D |
| Protocol version | 3 | Date and version identifier | 17 |
| Funding | 4 | Sources and types of financial, material, and other support | 24 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1, 24 |
| | 5b | Name and contact information for the trial sponsor | 24, Appendix D |
| | 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 | 24 |
| | 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) | Appendix A |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 5-7 |

| | | | |
|---|---|---|---|
| | 6b | Explanation for choice of comparators | NA |
| Objectives | 7 | Specific objectives or hypotheses | 7 |
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 8 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 9, Appendix A |
| 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) | 8, Table 1 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 11-12, Fig. 2 |
| | 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) | NA |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | 9 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 12 |
| Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 12 |
| Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 9-11, Fig. 1 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 15-16 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 9 |

**Methods: Assignment of interventions (for controlled trials)**

| | | | |
|---|---|---|---|
| Allocation: | | | |
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 10 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 10 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 10 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 10, 12 |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | NA |

**Methods: Data collection, management, and analysis**

| | | | |
|---|---|---|---|
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 12-15, Tables 2-3, Appendix B |
| | 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 | 9 |
| 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 | 15 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 15, 16 |

| | | | |
|---|---|---|---|
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 16 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 16 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | NA |
| | 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 | NA |
| 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 | 15 |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | NA |
| **Ethics and dissemination** | | | |
| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 17 |
| 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) | 17 |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 9 |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | NA |
| 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 | 15 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 24 |

| | | | |
|---|---|---|---|
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 15 |
| 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 | 15 |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 17 |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | 17 |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 17 |
| **Appendices** | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Appendix E |
| 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 | NA |

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

## Appendix D: World Health Organization Trial Registration Data Set

1. **Primary Registry and Trial Identifying Number:** ClinicalTrials.gov; NCT02421393; URL https://clinicaltrials.gov/ct2/show/NCT02421393.
2. **Date of Registration in Primary Registry:** 20 April. 2014
3. **Secondary Identifying Numbers:** NA.
4. **Source(s) of Monetary or Material Support:** Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: info@metabolicfitness.it.
5. **Primary Sponsor:** Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: info@metabolicfitness.it.
6. **Secondary Sponsor(s):** NA.
7. **Contact for Public Queries: Stefano Balducci, MD,** Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: sbalducci@esinet.it.
8. **Contact for Scientific Queries: Giuseppe Pugliese,** M.D., Ph.D., Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, Via di Grottarossa, 1035-1039 - 00189 Rome, Italy; Phone: +39-0633775440; Fax: +39-0633776327; E-mail: giuseppe.pugliese@uniroma1.it.
9. **Public Title:** The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes.
10. **Scientific Title:** The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes.
11. **Countries of Recruitment:** Italy.
12. **Health Condition(s) or Problem(s) Studied:** type 2 diabetes (T2D).
13. **Intervention:**
    a. Intervention arm
       - Name: Supervised exercise training.
       - Description: two weekly supervised mixed exercise training sessions for two years, on top of standard care.
    b. Standard care.
14. **Key Inclusion and Exclusion Criteria**
    a. Inclusion criteria: known T2D (defined by the ADA criteria) of at least 1-year duration. Additional requirements are age 40-80 years; BMI 27-40 kg/m$^2$; sedentary lifestyle (i.e., more than 8 hours/day spent in any waking behaviour characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture) and physically inactivity (i.e., insufficient amounts of PA according to current guidelines) from at least 6 months; a Short Battery Performance Test score ≥4; ability to walk 1.6 Km without assistance; and eligibility after cardiologic evaluation.
    b. Exclusion criteria: unable or unwilling to give informed consent or communicate with local study staff; current diagnosis of psychiatric disorder or hospitalization for depression in the past six months; self-reported alcohol or substance abuse within the past twelve months; self-reported

inability to walk two blocks; musculoskeletal disorders or deformities that may interfere with participation in the intervention; history of central nervous dysfunction such as hemiparesis; myelopathies; cerebral ataxia; clinical evidence of vestibular dysfunction; postural hypotension defined as a fall in BP when changing position of >20 mmHg (systole) or >10 mmHg (diastole); currently pregnant or nursing; cancer requiring treatment in the past five years, except for cancers that have clearly been cured or in the opinion of the investigator carry an excellent prognosis (e.g., stage 1 cervical cancer); chronic obstructive pulmonary disease; end-stage liver disease; chronic diabetic complications (recent major acute cardiovascular event, including heart attack, stroke/transient ischemic attack(s), revascularization procedure, or participation in a cardiac rehabilitation program within the past three months; pre-proliferative and proliferative retinopathy; macroalbuminuria and/or eGFR < 45 ml/min/1.73 $m^2$; severe motor and sensory neuropathy; diabetic foot with history of ulcer); cardiovascular disease at cardiologic examination (history of cardiac arrest; history of pulmonary embolism in the past six months; unstable angina pectoris or angina pectoris at rest; resting HR <45 beats/min or >100 beats/min; complex ventricular arrhythmia at rest or with exercise; uncontrolled atrial fibrillation with HR >100 beats/min; NYHA Class III or IV congestive heart failure; acute myocarditis; pericarditis or hypertrophic myocardiopathy; left bundle branch block or cardiac pacemaker); treatment with anti-fracture agents, oestrogens, aromatase inhibitors, testosterone, corticosteroids and/or glitazones; previous documented non-traumatic fractures; spinal deformity index (SDI) >3 (and >2 in a single vertebra); and a T score <-2.5 at spine/hip at DXA; haemoglobin (Hb) $A_{1c}$ >9.0%; blood pressure (BP) >150/90 mmHg; vitamin D <10 ng/ml; conditions not specifically mentioned above at the discretion of the clinical site.

15. **Study Type**

    a. Type of study: interventional.

    b. Study design:

    - Method of allocation: randomized
    - Masking: no (assessor-blinded)
    - Assignment: parallel
    - Purpose: testing the efficacy of a specific exercise training program in improving bone quality and strength in patients with T2D

    c. Phase: NA

    d. Allocation concealment mechanism and sequence generation: centralized randomization stratified by age, gender, and type of diabetes treatment (non-insulin versus insulin therapy), using a permuted-block randomization software which randomly varies the block size.

15. **Date of First Enrolment:** November 1, 2018 (expected).

16. **Target Sample Size:** 200

17. **Recruitment Status: recruiting.**

16. **Primary Outcome(s)**

    - Name: baseline to end-of-study change in Trabecular Bone Score (TBS);
    - Method of measurement: spine dual-energy X-ray absorptiometry (DXA)-derived software-based measure;
    - Time points: baseline and end-of-study.

17. **Key Secondary Outcomes**

a. Name: baseline to end-of-study change in broadband ultrasound attenuation (BUA), speed of sound (SOS), and quantitative ultrasound index (QUI); methods of measurement: quantitative ultrasound (QUS); time points: baseline and end-of-study.

b. Name: baseline to end-of-study change in multiple measured and calculated bone parameters; methods of measurement: peripheral quantitative computed tomography (pQCT); time points: baseline and end-of-study.

c. Name: baseline to end-of-study change in bone mineral density (BMD) and other DEXA-derived measures; method of measurement: spine and hip DXA; time points: baseline and end-of-study.

d. Name: baseline to end-of-study change in markers of bone turnover; method of measurement: immunochemical methods; time points: baseline and end-of-study.

e. Name: baseline to end-of-study change in body composition; method of measurement: total body DXA; time points: baseline and end-of-study.

f. Name: baseline to end-of-study change in muscle strength; methods of measurement: isometric muscle strength test; time points: baseline and end-of-study.

g. Name: baseline to end-of-study change in muscle cross-sectional area; method of measurement: pQCT; time points: baseline and end-of-study.

h. Name: baseline to end-of-study change in balance, gait and power; method of measurement: Short Battery Performance Test; time points: baseline and end-of-study.

i. Name: number of falls; 17-item History of Falls questionnaire; time points: baseline and every 6 months thereafter for 7 years (2-year trial + 5-year post-trial follow-up).

j. Name: symptomatic fractures; method of measurement: clinical and radiographic records; time points: baseline and every 6 months thereafter for 7 years (2-year trial + 5-year post-trial follow-up).

k. Name: asymptomatic and symptomatic fractures; method of measurement: vertebral morphometry; time points: baseline and every 6 months thereafter for 7 years (2-year trial + 5-year post-trial follow-up).

## Appendix E: Informed consent

### Patient Information Sheet

### TITLE OF THE STUDY: The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes

This study is registered at ClinicalTrial.gov as "Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET-BONE) in type 2 diabetes: an exercise intervention program for reducing the risk of fractures "(N. NCT02421393, URL https://clinicaltrials.gov/ct2/show/NCT02421393)

**IDENTIFICATION OF THE STUDY**

Dear Sir/Madam,

The study, which your physician (diabetes specialist) is inviting you to participate in, aims to evaluate the effect of 2-year training consisting of supervised and combined aerobic and resistance exercise sessions in individuals with type 2 diabetes mellitus on:

- bone quality and mass;
- bone metabolism;
- muscle strength and mass;
- balance and gait;
- falls;
- symptomatic and asymptomatic fractures.

The hypothesis is that a specific exercise training program produces a significant improvement in the qualitative and quantitative bone parameters by influencing bone metabolism, with a consequent reduction in the risk of fractures and, in the long term, a significantly reduced number of fractures.

The research involves about 200 patients with type 2 diabetes.

During the study you will be assigned to one of the following two groups:

1. Exercise (EXE) group, which receives standard care and participates in two weekly mixed exercise training sessions for two years, supervised by an exercise specialist at the Metabolic Fitness Association.
2. Control (CON) group, which receives only standard care.

The study will have a duration of 2 years plus a 5-year post-trial follow-up, during which you will be subjected to 6-month monitoring visits. The parameters reported above will be assessed at the beginning and at the end of the 2-year period, except for falls and fractures, which will be assessed every 6 months for the entire 7 year period (2-year trial + 5-year post-trial follow-up).

**PROTECTION OF PERSONAL DATA:**

- All information concerning you, the collection and processing of which is connected and indispensable to the achievement of the objectives of this study, will be treated in a manner suitable to ensure

absolute confidentiality and security in accordance with the provisions for the protection of personal data and the right to privacy (Italian Data Protection Act, No. 675 of December 6, 1996 and subsequent amendments/additions).

- You will be identified by a code and the clinical information concerning you will not be disclosed without your written permission. The data collected will consist of your initials, date of birth, sex and otherwise sensitive clinical data as suitable to reveal your state of health.
- As a participant in the processing of your personal data, you will have full access, through your family doctor, to the information concerning you. You will also have the right to exercise all the rights of cancellation, transformation, integration, updating, correction and blocking of your data within the limits set out in art. 13 of the Italian Data Protection Act 675/96 mentioned above. You will not be charged any fee for the scheduled exams, the results of which will be promptly communicated to your family doctor.

**STUDY BENEFITS:**

- Upon agreeing to participate in this study, you might be assigned to follow a supervised exercise training program. Whatever group you are assigned to, you will be under strict control by a staff-member, medical or otherwise, specialized in the management of type 2 diabetes, including physical activity/exercise therapy.
- In addition, your doctor may become aware of the presence of cardiovascular risk factors or complications to be monitored.
- Finally, the knowledge acquired thanks to your participation will be useful both for you and for other patients.

**PARTICIPATION IN THE STUDY:**

- Your participation in this study is completely cost-free and, if you decide not to take part, you will still be assisted in the most appropriate medical treatment.
- We invite you to ask your family physician any question you deem appropriate. Your doctor will also ask you to sign and date the consent form for the processing of personal data to confirm that you have read all the information contained herein, which includes that you have understood the aims of the study and most importantly, that you have freely given your consent to the collection and processing of your personal data.

## Patient Consent Form

**TITLE OF THE STUDY: The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes**

I, the undersigned _____ born in _____ on _____

and resident in _____

hereby declare, after reading the information, the following:

- to have read and understood the patient information sheet of the aforementioned study and to have had ample time and opportunity to ask questions and obtain satisfactory answers to the investigator;
- to have understood that my participation is voluntary and that I can withdraw from the study at any time, without having to explain or influence any future medical assistance in any way;
- to have understood that my personal data will be processed according to the regulations in force specified in the information sheet of the study and that I can exercise my rights by contacting the Data Controller at any time and in the manner specified in accordance with art. 7, Legislative Decree n. 196 of 30/06/2003, (so-called Privacy Code).

Following these statements, I declare that I, the undersigned, freely:

- accept to participate in the study mentioned above;
- consent to the processing of personal and sensitive data collected in the context of this study, in the terms and methods indicated and explained in the information, aware that anonymity in the treatment will be guaranteed;
- consent that the investigator and his collaborators, as expressly indicated in the informative report, collect and process the data deriving from the investigations for the express purpose of a scientific publication.

**Signature of the patient** _____ **date** _____

**Surname and name of the patient** _____

**Signature of the investigator** _____ **date** _____

**Surname and name of the investigator** _____

BMJ Open

# Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes: Study protocol for a randomized clinical trial

| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2018-027429.R2 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 13-Sep-2019 |
| Complete List of Authors: | Balducci, Stefano; La Sapienza University, Department of Clinical and Molecular Medicine<br>Conti, Francesco; La Sapienza University, Rome, Department of Clinical and Molecular Medicine<br>Sacchetti, Massimo; Foro Italico University, Department of Human Movement and Sport Sciences<br>Russo, Cosimo; Metagym<br>Argento, Giuseppe; Sant'Andrea University Hospital, Radiology Unit<br>Haxhi, Jonida; La Sapienza University, Department of Clinical and Molecular Medicine<br>Orlando, Giorgio; Foro Italico University, Department of Human Movement and Sport Sciences<br>Rapisarda, Gianvito; La Sapienza University, Department of Clinical and Molecular Medicine<br>D'Errico, Valeria; La Sapienza University, Department of Clinical and Molecular Medicine<br>Cardelli, Patrizia; La Sapienza University, Department of Clinical and Molecular Medicine<br>Pugliese, Luca; Sant'Andrea University Hospital, Radiology Unit<br>Laghi, Andrea; Sant'Andrea University Hospital, Radiology Unit<br>Vitale, Martina; La Sapienza University, Department of Clinical and Molecular Medicine<br>Bollanti, Lucilla; La Sapienza University, Department of Clinical and Molecular Medicine<br>Zanuso , S; Coventry University, Centre for Applied Biological & Exercise Sciences<br>Nicolucci, Antonio; Center for Outcomes Research and clinical Epidemiology (CORE), Pescara, Italy.,<br>Pugliese, Giuseppe; La Sapienza University, Rome, Department of Clinical and Molecular Medicine |
| Primary Subject Heading: | Diabetes and endocrinology |
| Secondary Subject Heading: | Sports and exercise medicine |
| Keywords: | General diabetes < DIABETES & ENDOCRINOLOGY, Diabetes & endocrinology < INTERNAL MEDICINE, Bone diseases < ORTHOPAEDIC & TRAUMA SURGERY, PREVENTIVE MEDICINE |

# Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes: Study protocol for a randomized clinical trial

Stefano Balducci [1,2,3], Francesco G. Conti [1,2], Massimo Sacchetti [4], Cosimo R. Russo [5], Giuseppe Argento [6], Jonida Haxhi [2,3,4], Giorgio Orlando [4*], Gianvito Rapisarda [2,3], Valeria D'Errico [2,3], Patrizia Cardelli [1,7], Luca Pugliese [6†], Andrea Laghi [6,8], Martina Vitale [1,2], Lucilla Bollanti [1,2], Silvano Zanuso [9], Antonio Nicolucci [10], and Giuseppe Pugliese [1,2]; for the SWEET BONE Investigators [‡].

[1] Department of Clinical and Molecular Medicine, ''La Sapienza'' University, Rome, Italy; [2] Diabetes Unit, Sant'Andrea University Hospital, Rome, Italy; [3] Metabolic Fitness Association, Monterotondo, Rome, Italy; [4] Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy; [5] Metagym Fitness Centre, Florence, Italy; [6] Radiology Unit, Sant'Andrea University Hospital, Rome, Italy; [7] Laboratory of Clinical Chemistry, Sant'Andrea University Hospital, Rome, Italy; [8] Department of Radiological Sciences, Oncology and Pathology, ''La Sapienza'' University, Rome, Italy; [9] Centre for Applied Biological & Exercise Sciences, Faculty of Health & Life Sciences, Coventry University, Coventry, UK; and [10] Centre for Outcomes Research and Clinical Epidemiology (CORESEARCH), Pescara, Italy.

Current address: [*] School of Healthcare Science, Faculty of Science & Engineering, Manchester Metropolitan University, Manchester, UK; [†] Department of Diagnostic Imaging, "Tor Vergata" University, Rome, Italy.

[‡] See Appendix A for the complete list of the SWEET_BONE Investigators.

**Corresponding Author:** Giuseppe PUGLIESE, MD, PhD, Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, Via di Grottarossa, 1035-1039 - 00189 Rome, Italy; Phone: +39-0633775440; Fax: +39-0633776327; E-mail: giuseppe.pugliese@uniroma1.it.

**Key words:** type 2 diabetes; bone quality; bone mass; bone fractures; exercise; physical fitness.

**Word count:** abstract 300; text 4,000.

## Abstract

**Introduction.** Type 2 diabetes (T2D) is associated with an increased fracture risk despite normal-to-increased bone mineral density, suggesting reduced bone quality. Exercise may be effective in reducing fracture risk ~~in~~ by ameliorating muscle dysfunction and reducing risk of fall, though it is unclear whether it can improve bone quality.

**Methods and analysis.** The "Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in T2D" is an open-label, assessor-blinded, randomized clinical trial comparing an exercise training program of 2-year duration, specifically designed for improving bone quality and strength, with standard care in T2D individuals. Two-hundred T2D patients aged 65-75 years will be randomized 1:1 to supervised exercise training or standard care, stratified by gender, age ≤ or >70 years, and non-insulin or insulin treatment. The intervention consists of two weekly supervised sessions, each starting with 5 min of warm-up, followed by 20 min of aerobic training, 30 min of resistance training, and 20 min of core stability, balance, and flexibility training. Participants will wear weighted vests during aerobic and resistance training. The primary endpoint is baseline to end-of-study change in trabecular bone score, a parameter of bone quality consistently shown to be reduced in T2D. Secondary endpoints include changes in other potential measures of bone quality, as assessed by quantitative ultrasound and peripheral quantitative computed tomography; bone mass; markers of bone turnover; muscle strength, mass, and power; balance and gait. Falls and asymptomatic and symptomatic fractures will be evaluated over 7 years, including a 5-year post-trial follow-up. The superiority of the intervention will be assessed by comparing between-groups baseline to end-of-study changes.

**Ethics and dissemination.** This study was approved by the institutional ethics committee. Written informed consent will be obtained from all participants. The study results will be submitted for peer-reviewed publication.

**Registration details.** ClinicalTrials.gov, NCT02421393.

## Strengths and limitations of this study

- This is the first study investigating whether a specifically designed exercise training program of 2-year duration is effective in improving bone quality and strength in patients with type 2 diabetes, thus reducing the increased fracture risk characterizing these individuals.
- A wide range of parameters of bone quality and strength is assessed, together with measures of bone mass and muscle mass, strength and power, which all may affect fracture risk, and falls and fractures over an extended 7-year period.
- All the physicians, exercise specialists, and outcome assessors have been specifically trained for conducting this trial and participated in a pilot study aimed at setting up the trial protocol.
- There are no data on the effect of exercise on the primary endpoint trabecular bone score, a surrogate measure of bone quality.
- Generalizability and implementation in clinical practice of this approach will require further investigation and validation in different cohorts or contexts.

## Introduction

Risk of fracture is significantly increased in type 1 diabetes (T1D) and, to a lower extent, in type 2 diabetes (T2D).[1, 2] Nevertheless, bone mineral density (BMD) was reported to be normal or even increased in T2D patients, whereas it was found almost consistently reduced in T1D individuals.[1] Notably, in T2D patients, the increase in fracture risk remained after adjustment for BMD [3-5] and also for falls,[3, 4, 6] which are more frequent in older individuals with T2D than in those without.[7] In addition, as compared with non-diabetic individuals, patients with T2D have a higher T-score for a similar fracture risk.[8] While the preserved bone mass may account for the lower fracture risk in T2D versus T1D, a reduced bone quality has been claimed to explain the discrepancy between normal BMD and increased fracture risk in T2D patients.[1, 2]

Bone quality is determined by (a) bone architecture, including geometry (macro-architecture) and micro-architecture; and (b) material properties, including mineralization and collagen cross-links, which in turn are influenced by bone turnover as well as by accumulation of microdamage and microstructural discontinuities.[9, 10] While conventional dual-energy X-ray absorptiometry (DXA) measures bone mass, several techniques have been proposed for non-invasive assessment of bone quality.[11, 12] The trabecular bone score (TBS) is a gray-level texture measurement based on 2D projection images acquired during a DXA lumbar spine scan.[13] It was consistently found to be reduced in T2D patients with fracture versus those without [14] and in T2D versus non-diabetic individuals,[15-20] and to predict fracture risk independently of BMD.[15] Quantitative ultrasound (QUS) provides an estimate of BMD,[21] which predicted fracture risk better than DXA-derived BMD in older women with T2D;[22] in addition, QUS evaluates micro-architecture and material properties.[23, 24] However, QUS-derived bone structure measures were not consistently lower in T2D patients with versus those without fracture [25, 26] and in T2D versus non-diabetic individuals.[27, 28] In addition to volumetric BMD (vBMD), low- and high-resolution peripheral quantitative computed tomography (pQCT) provides measures of bone geometry and architecture.[11]. Higher cortical porosity and lower calculated strength were reported in T2D patients with versus those without fracture [29, 30] and in T2D versus non-diabetic individuals.[31-34]

Physical activity (PA)/exercise has been suggested as an effective tool for improving bone health in individuals at high fracture risk. It is known that both compressive loading from weight bearing and muscle contraction deform the osteocytes, which function as strain transducers by signalling osteoblasts, osteoclasts, and other cells to produce or break down bone.[35, 36] Appropriate types, amounts, and directions of strain result in bone mass maintenance, bone formation, and/or changes in bone geometry that improve bone strength.[37]

Exercise was shown to improve BMD to a relatively small, but clinically significant extent.[38] There is also a great deal of evidence from observational studies that higher PA levels are associated with fewer fractures in community-dwelling populations,[39] and postmenopausal women who performed spinal extension exercises showed a lower incidence of vertebral fractures.[40] Combination of diet and exercise was shown to provide greater improvement in physical function than either intervention alone.[41] Moreover, exercise training prevented the increase in bone turnover and attenuated the decrease in hip BMD associated with diet-induced weight loss,[42] and resistance exercise attenuated diet-induced decrease in muscle mass and BMD more than aerobic training.[43] Resistance exercise was also shown to decrease falls and risk of falls, especially when focused on strengthening the hip and ankle muscles involved in balance maintenance.[44]

These observations indicate that PA/exercise, especially of resistant type, may be effective in reducing fracture risk in T2D patients by ameliorating muscle mass, strength, and quality [45] and reducing falls and risk of fall.[7] However, it is unclear whether PA/exercise may reduce fracture risk also by directly improving bone health in individuals with preserved BMD, such as those with T2D. Indeed, to date, there are no data on whether exercise training is effective in ameliorating bone quality and whether improved quality results in increased bone strength and reduced fracture risk in patients with T2D.

The “Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in T2D” is aimed at investigating the efficacy of a specific exercise intervention program of 2-year duration on parameters of bone quality and strength in patients with T2D.

## Methods and analysis

### Trial design

The SWEET BONE is an open-label, assessor-blinded, parallel, superiority randomized clinical trial (RCT) comparing a specifically designed exercise intervention program with standard care in T2D individuals. The trial flow chart is shown in Figure 1.

### Participants

This study will enrol patients with T2D (defined by the American Diabetes Association criteria [46]) of ≥5-year duration, of both sexes, aged 65-75 years. Additional requirements will be: physically inactivity (i.e. insufficient amounts of PA according to current guidelines) [47] and sedentary lifestyle (i.e. ≥8 hours/day spent in a sitting or reclining posture) [48] from ≥6 months; body mass index (BMI) 27-40 kg/m$^2$; ability to walk 1.6 Km without assistance; a Short Battery Performance Test score ≥4; and eligibility after cardiologic evaluation.

The criteria listed in Table 1 will be used to exclude individuals with conditions limiting or contraindicating PA, reduce lifespan, and affect conduct of the trial and/or the safety of intervention. Among exclusion criteria there are treatment with anti-fracture agents, oestrogens, aromatase inhibitors, testosterone, corticosteroids and/or glitazones; previous documented non-traumatic fractures; spinal deformity index (SDI) >5 (>2 in a single vertebra); and a T score <-2.5 at DXA. Individuals with haemoglobin (Hb) $A_{1c}$ >9.0%, blood pressure (BP) >150/90 mmHg, and/or vitamin D <10 ng/ml will be re-evaluated for eligibility after appropriate treatment.

A sample of 50 non-diabetic individuals meeting the inclusion/exclusion criteria reported above (except for T2D-related criteria) and matched 1:4 by age, gender, and BMI will serve as controls for baseline measures.

### Investigators

All the SWEET-BONE physicians, exercise specialists, and outcome assessors (see Appendix A) have been specifically trained for conducting this RCT and participated in a pilot study aimed at setting up the trial protocol.

To minimize dropout and reduce the attrition bias due to missing data, both physicians and exercise specialists have been instructed on how to promote participant retention in the trial, i.e., contact participants at regular intervals, keep up to date contact information for participants, and collect complete data for the primary and secondary outcomes, regardless of whether individuals continue to receive the assigned intervention.

**Recruitment**

Starting on 1 November, 2018, 200 patients will be recruited at the Diabetes Unit of Sant'Andrea University Hospital, a tertiary referral, outpatients Diabetes Clinic in Rome, Italy. All patients attending the clinic will be evaluated for eligibility. The recruitment process will include four visits designated as R1, R2, R3, and R4.

On R1, eligible patients will be identified based on medical history, clinical examination, and results of the Minnesota leisure-time PA questionnaire. Then, patients will be asked to sign an informed consent and will be registered in the SWEET-BONE database available at http://www.metabolicfitness.it/. Finally, patients will undergo a cardiologic examination, including a resting electrocardiogram (ECG) and, based on clinical judgment, an echocardiogram and/or an ECG treadmill test.

On R2, baseline anthropometrical and clinical parameters and blood and urine samples for biochemical testing will be taken. Subsequently, participants will perform a Short Battery Performance Test and undergo measurement of ankle-brachial index and fundus evaluation. Finally, patients will attend a run-in session for familiarization with testing devices and protocols for the assessment of physical fitness.

On R3, patients will be asked to fill in the History of Falls questionnaire, the Physical Activity Scale for the Elderly (PASE) questionnaire, and a questionnaire for musculoskeletal (MS) symptoms. Then, participants will undergo x-ray of dorso-lumbar spine for vertebral morphometry, and total body and segmental DXA. Finally, they will attend another run-in session for familiarization prior to the assessment of physical fitness.

On R4, patients will be prescribed a standard treatment regimen. Then, they will undergo the following procedures: peripheral QCT (pQCT), calcaneal QUS, and dynamometry. Finally, patients will be subjected to the assessment of physical fitness and will be informed about group assignment.

**Randomization**

Patients will be randomized 1:1 to supervised exercise training on top of standard care (exercise, EXE, group; n=100) versus standard care (control, CON, group; n=100) for 24 months.

Randomization will be stratified by gender (males versus females), age (65-70 versus 71-75 years) and type of diabetes treatment (non-insulin versus insulin), using a permuted-block randomization software which randomly varies the block size. To ensure allocation concealment, randomization will be centralized at the Centre for Outcomes Research and Clinical Epidemiology (CORESEARCH), and the group assignment of newly recruited patients will be communicated to the investigators by telephone call.

After randomization, participants, physicians and exercise specialists will not be blinded to group assignment, as blinding in unfeasible in exercise intervention studies.

**Follow-up**

Participants from both groups will attend four follow-up visits, designated as F1, F2, F3, and F4, at month 6, 12, 18, and 24, respectively.

On F1, F2, and F3, patients will undergo a routine diabetes visit, with eventual adjustment of dietary and pharmacological prescriptions, and will be asked to fill in the History of Falls, PASE, and MS questionnaires.

On F4, end-of study anthropometrical and clinical parameters and blood and urine samples for biochemical testing will be taken. Then, participants will be asked to fill in the History of Falls, PASE, and MS questionnaires and to perform a Short Battery Performance Test. On different days, patients will undergo x-ray of dorso-lumbar spine for vertebral morphometry, total body and segmental DXA, pQCT, calcaneal QUS, dynamometry, and assessment of physical fitness.

**Post-trial follow-up**

Participants will be followed every 6 months for additional 5 years for routine diabetes visits. On these occasions, they will be asked to provide clinical records on eventual fractures, to fill in the History of Falls,

PASE, and MS questionnaires, and to perform a Short Battery Performance Test. At the end of the 5-year post-trial follow-up, participants will undergo vertebral morphometry to detect asymptomatic fractures.

**Intervention**

The training program for the EXE group will consist of two 75-min weekly sessions, supervised by an exercise specialist in the gym facility of the Metabolic Fitness Association (Figure 2). We conducted a pilot study on a small sample of T2D patients meeting the inclusion/exclusion criteria for this RCT in order to set up the training program.

Each session will start with 5 min of warm up, followed by 20 min of aerobic training using treadmill. Then, patients will perform 30 min of resistance (strength and power) training consisting of 6 resistance exercises using machines and targeting muscle groups influencing body regions which are sites of fragility fractures (15 min) and 3 20-repetition series of 3 different "weight bearing" exercises (15 min). The session will end with 20 min of core stability training (8 min), which improves the ability to control the position and movement of the central portion of the body and targets the deep abdominal muscles that assist in posture maintenance and arm and leg movements, followed by balance (8 min) and flexibility (4 min) training.

The exercise intensity and difficulty level will be increased gradually in order to ensure safety and prevent attrition, as shown in a previous RCT [49] and confirmed by the pilot study. In particular, the intensity of aerobic and resistance exercise will be increased from light to moderate and adjusted according to improvements in physical fitness. The velocity of execution of resistance exercises during the concentric phase of the movement, the impact, height of jumps and amplitude of movements of weight bearing exercises, and the difficulty level of balance training will be also progressively increased.

Starting at month 2, a weighted vest will be worn during each session (while performing aerobic training and weight bearing exercises), and also outside the sessions (for at least 1 hour and during 3 10-repetition series of step-up and sit-to-stand in three non-training days every week). Patients will be asked to record in a daily diary the time spent wearing the weighted vest outside the sessions. Weight of vests will be 2% of body weight and will be increased by 2% every 6 months (i.e., up to 8%).

**Standard care**

All patients will be subjected to a treatment regimen aimed at achieving glycaemic, lipid, BP and body weight targets, as established by current guidelines and including nutritional therapy and glucose-, lipid- and BP-lowering agents as needed.[46] Vitamin D will be supplemented to maintain levels higher than 30 ng/ml.

At intermediates routine diabetes visits, drugs will be adjusted to attain target levels, following a pre-specified algorithm, and changes will be recorded into the SWEET-BONE database.

**Outcomes**

The primary endpoint is baseline to end-of-study change in TBS, based on previous reports showing that it is consistently lower in T2D versus non-diabetic individuals [15-20].

Secondary endpoints include (a) other potential measures of bone quality, as assessed by QUS and pQCT; (b) bone mass (BMD); (c) markers of bone turnover; (d) body composition; (e) muscle strength, mass, and power; (f) balance and gait; (g) number of falls; and (h) asymptomatic and symptomatic fractures. Falls and fractures will be evaluated over 7 years (i.e., including the 5-year post-trial follow-up).

PA level, cardio-respiratory fitness, flexibility, MS symptoms, modifiable cardiovascular risk factors, medications, and coronary heart disease (CHD) and stroke 10-year risk scores will be also evaluated.

The assessors of outcome measures will be blinded to group assignment.

**Measurements**

***Bone mass and quality.*** Bone mass will be assessed by DXA scans of the lumbar spine (L1 to L4) and total femur using Hologic QDR 4500 W 2000 (Hologic, Bedford, MA, USA). Areal BMD (aBMD, g/cm$^2$) in the lumbar spine and femoral neck will be recorded and the corresponding T scores and Z scores will be obtained. Composite indices of femoral neck strength will be also computed, as previously reported.[50] TBS will be then measured using the Hologic TBS Insight software (Hologic). Calcaneal QUS measurements will be performed using the Sahara® Clinical Bone Sonometer (Technologic, Turin, Italy). Broadband ultrasound attenuation (BUA; dB/MHz) and speed of sound (SOS; m/s) will be measured, and the quantitative ultrasound index (QUI) will be

then calculated. BMD will be also estimated from QUS measurements (eBMD, g/cm$^2$). Bone density and macroarchitecture will be evaluated using an XCT-2000 pQCT scanner (Norland Stratec, Stratec, Pforzheim, Germany).[51] Slices (2.5 mm) will be obtained at the 4%, 14%, 38%, and 66% sites of the left tibia and at the 4% site of the nondominant radius. At the metaphyses (4% site) of tibia and radius, total vBMD (Tot-vBMD) and Trabecular (Trab)-vBMD (mg/cm$^3$) will be measured. At the 14% site of the tibia, cortical bone area (Cort-A, mm$^2$) and cortical bone mineral content (Cort-BMC, mg/cm), two markers of resistance to compressive and tensile loads, will be measured, and the section modulus will be calculated from the antero-posterior, latero-lateral, and polar moments of inertia (Ix, Iy, and Ip, respectively) and used to obtain the stress-strain index (SSI, mm$^3$), a surrogate measure of resistance to bending (xSSI and ySSI) and torsional (pSSI) loads. At the 38% site of the tibia, cortical vBMD (Cort- vBMD, mg/cm$^3$) and total cross-sectional area (Tot-CSA, mm$^2$) will measured, together with calculation of cortical thickness (CT, mm) and circularity index (CI), a proxy of tibial geometrical load adaptation.

***Markers of bone turnover.*** Serum calcium and phosphorus, 25OH Vitamin D, and parathyroid hormone will be measured, together with the following markers of bone turnover: total and bone-specific alkaline phosphatase, osteocalcin, and procollagen I intact N-terminal, for bone formation, and C-terminal telopeptide of type I collagen, tartrate-resistant acid phosphatase 5b, sclerostin, and Dickkopf-1, for bone resorption. These measurements will be centralized at the Laboratory of Clinical Chemistry of Sant'Andrea University Hospital, using the methods reported in Table 2.

***Body composition.*** Total body DXA will be used to evaluate body composition, with measurement of total body lean mass and total body fat mass.

***Muscle strength.*** Isometric muscle strength will be also assessed by means of a strain gauge tensiometer (Digimax, Mechatronic GmbH, Germany), as previously reported.[52] Maximal voluntary contractions are performed at the shoulder press (Technogym, Gambettola, Italy) along the sagittal plan, with a 45° and 90° angle at the elbow and between the upper arm and the trunk, respectively, for the upper body, and at the leg

extension machine (Technogym), with a 90° angle at the knee and the hip, for the lower body. Values will be expressed in Nm for two arms.

***Muscle cross-sectional area.*** The cross-sectional areas of muscles of the leg will be measured by pQCT at the 66% site of the tibia at the end of bone assessments.[53]

***Physical fitness.*** Physical fitness will be evaluated at baseline, end-of-study and, in the EXE group, also at month 6, 12, and 18, in order to adjust training loads. Cardio-respiratory fitness, muscle fitness, and flexibility will be assessed by a sub-maximal evaluation of oxygen consumption at 80% of the maximal heart rate to predict maximal oxygen consumption ($VO_{2max}$), a maximal repetition (or 5-8 RM) to predict one-repetition maximum (1-RM), and a standard bending test, respectively, as previously reported.[49, 54]

***Balance, gait and power.*** A "Short Battery Performance Test" will be performed for the assessment of balance (side-by-side stand, semi-tandem stand and tandem stand), gait (gait speed test) and power (chair stand test).[55]

***Number of falls.*** Falls will be recorded using the 17-item History of Falls questionnaire (see Appendix B1).[56]

***Symptomatic and asymptomatic fractures.*** Patients will be interviewed to record symptomatic fractures, which will be adjudicated based on clinical and radiographic records. Asymptomatic fractures will be identified by vertebral morphometry.

***PA level.*** The level of PA will be evaluated throughout the study by asking patients to fill in the PASE questionnaire (see Appendix B2), a validated instrument for the measurement of PA level in individuals aged ≥65 years.[57] The amount of supervised exercise in the EXE group will be measured as previously reported.[49, 54]

***MS symptoms.*** MS symptoms will be evaluated by a 50-item self-report questionnaire (see Appendix B3).[58]

***Cardiovascular risk factors and scores.*** The BMI will be calculated from body weight and height, while waist circumference will be taken at the umbilicus and BP will be recorded with a sphygmomanometer after a five-

minute rest with the patient seated. Blood and urine samples will be taken for measuring the biochemical parameters reported in Table 3 at the Laboratory of Clinical Chemistry of Sant'Andrea University Hospital. Global and fatal CHD and stroke 10-year risk scores will be calculated using the United Kingdom Prospective Diabetes Study (UKPDS) risk engine.[59] Cardiovascular risk factors and scores will be assessed at baseline and end-of-study.

**Adverse events**

Adverse events will be reported at intermediate visits and, for EXE participants, also at supervised sessions, by completing a standard form.

The risk of injuries and other adverse events during the training sessions will be covered by an insurance (N. 390-01583709-14010, HDI-Gerling Industrie Versicherung AG, Leipzig, Germany).

**Data collection, storage and security**

Data collected into the SWEET-BONE database will be saved to a password-protected server in the Metabolic Fitness Association and accessed only by members of the research team.

Once uploaded to the server, data will be securely deleted from the recording devices. Patient questionnaire data will be made anonymous and stored in locked filing cabinets.

**Statistical analysis**

Sample size calculation was based on our pilot study showing that TBS was 1.225±0.085 (SD) in T2D individuals. To detect a between-group difference of 0.045 in TBS (i.e., effect size=0.50) with statistical power of 90% ($\alpha$=0.05) by two-sided two-sample equal-variance t-test, 86 patients per arm are needed. A sample of 200 patients allows to tolerate a 14% dropout rate.

The $\chi^2$ or, where appropriate, the Fisher's exact test, for categorical variables, and the Student's t test or the corresponding nonparametric Mann-Whitney test for continuous variables will be utilized to compare patients' characteristics at baseline. The intention-to-treat analysis will be applied to all randomized patients. The superiority of the intervention on the primary and secondary endpoints will be assessed by mixed models for

repeated measures. Pre-specified subgroup analyses will be conducted by gender, age (65-70 versus 71-75), and type of diabetes treatment (non-insulin versus insulin).

To account for change in medication throughout the study period, which might affect bone parameters, we will perform both multiple regression and sensitivity analyses. In the regression models, the dependent variable will be represented by baseline to end-of-study changes. Treatment at baseline and treatment initiation during the study will be included in the model as dichotomous variables (yes versus no), whereas drug dosage will be not taken into consideration. Sensitivity analysis will be conducted by comparing study arms after exclusion of patients who modified treatment.

Repeated measures models with an autoregressive correlation type matrix make an assumption of missing at random and account for both missingness at random and potential correlation within participants, as they allow evaluating all individuals, including those with incomplete data.[60] Finally, to guarantee replicability and avoid outcome selective reporting, a fully specified statistical analysis plan will be written before unmasking.

Statistical analyses will be performed by at the CORESEARCH using SAS software release 9.3 (Cary, NC, USA) and the statistical significance level will be set at $\alpha < 0.05$ (2-tailed). Because of the potential for type 1 error due to multiple comparisons, findings for analyses of secondary endpoints should be interpreted as exploratory.

**Patient and public involvement**

Patients or public will not be involved in the study, except for the burden of the intervention, which will be assessed by patients themselves and reported to the exercise specialist at each session, in order to identify the appropriate training modalities to minimize the risk of injury or adverse events.

## Ethics and Dissemination

The research protocol (version #3, February 28, 2013), which follows the SPIRIT guideline, complies with the Declaration of Helsinki. It has been approved by the Ethics Committee of Sant'Andrea University Hospital on 21 March, 2013 (Prot. n. 2583/2013) and has been registered with ClinicalTrials.gov on 20 April. 2014 (NCT02421393; URL https://clinicaltrials.gov/ct2/show/NCT02421393) (see Appendix C). Important protocol amendments (e.g., changes to eligibility criteria, outcomes, analyses) will be communicated to relevant parties, i.e., investigators, trial participants, trial registry, and the Ethics Committee.

All participants will provide written informed consent (see Appendix D) following verbal and written explanation of the study protocol and the opportunity to ask questions. Participants will not be provided with an honorarium and will be free to withdraw from the trial at any time without prejudice to future treatment.

To the best of our knowledge, the SWEET-BONE is the first study investigating whether a specifically designed exercise training program is effective in improving bone quality and strength in patients with T2D, thus potentially reducing the increased fracture risk characterizing these individuals despite preserved bone mass. The beneficial effects on bone quality would be additional to those on muscle strength and mass and risk of fall, which may reduce per se the risk of fracture. Potential pitfalls include the lack of data on TBS change over time in T2D individuals and the impact of exercise on this surrogate measure of bone quality. However, an age-dependent reduction in TBS of up to 0.5%/year has been reported in the general population [61-64] and such decrease is likely to be accelerated in T2D patients, given the large reduction in TBS detected in T2D versus non-diabetic individuals.[15-20, 65] In addition, in osteoporotic individuals, TBS was shown to be markedly increased (by ~4% in 2-to-3 years) by osteoanabolic agents such as teriparatide, though less than spine BMD,[66-68] whereas antiresorptive agents, which merely increase bone mineralization, were virtually ineffective.[64] Therefore, exercise, by virtue of its potential osteoanaboolic effect, is likely to influence positively TBS,[69] consistent with a recent cross-sectional study showing that people with higher levels of

objectively measured PA had higher TBS (and BMD).[70] Finally, generalizability and implementation in clinical practice of this approach will require further investigation and validation in different cohorts or contexts.

Results will be presented at scientific meetings and published in peer-reviewed journals. All publications and presentations related to the study will be authorized and reviewed by the study investigators. The ICMJE Recommendations will be adopted for authorship [71].

After publication of results, public access to the full protocol, participant-level dataset, and statistical code will be eventually granted upon request.

## References


1. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. *Osteoporos Int.* 2007;18:427–444.
2. Janghorbani M, Van Dam RM, Willett WC, et al. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. *Am J Epidemiol.* 2007;166:495–505.
3. Schwartz AV, Sellmeyer DE, Ensrud KE, et al. Older women with diabetes have an increased risk of fracture: a prospective study. *J Clin Endocrinol Metab.* 2001;86:32–38.
4. Strotmeyer ES, Cauley JA, Schwartz AV, et al. Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: The Health, Aging, and Body Composition Study. *Arch Intern Med.* 2005;165:1612–1617.
5. Napoli N, Strotmeyer ES, Ensrud KE, et al. Fracture risk in diabetic elderly men: the MrOS study. *Diabetologia.* 2014;57:2057–2065.
6. Bonds DE, Larson JC, Schwartz AV, et al. Risk of fracture in women with type 2 diabetes: the Women's Health Initiative Observational Study. *J Clin Endocrinol Metab.* 2006;91:3404–3410.
7. Schwartz AV, Hillier TA, Sellmeyer DE, et al. Older women with diabetes have a higher risk of falls: a prospective study. *Diabetes Care.* 2002;25:1749–1754.
8. Schwartz AV, Vittinghoff E, Bauer DC, et al. Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. *JAMA.* 2011;305:2184–2192.
9. Fonseca H, Moreira-Gonçalves D, Coriolano HJ, et al. Bone quality: the determinants of bone strength and fragility. *Sports Med.* 2014;44:37–53.
10. Farr JN, Khosla S. Determinants of bone strength and quality in diabetes mellitus in humans. *Bone.* 2016;82:28–34.
11. Rubin MR, Patsch JM. Assessment of bone turnover and bone quality in type 2 diabetic bone disease: current concepts and future directions. *Bone Res.* 2016;4:16001.

12. Jiang N, Xia W. Assessment of bone quality in patients with diabetes mellitus. *Osteoporos Int.* 2018;29:1721–1736.

13. Bousson V, Bergot C, Sutter B, et al. Trabecular bone score (TBS): available knowledge, clinical relevance, and future prospects. *Osteoporos Int.* 2012;23:1489–1501.

14. Zhukouskaya VV, Eller-Vainicher C, Gaudio A, et al. The utility of lumbar spine trabecular bone score and femoral neck bone mineral density for identifying asymptomatic vertebral fractures in well-compensated type 2 diabetic patients. *Osteoporos Int.* 2016;27:49–56.

15. Leslie WD, Aubry-Rozier B, Lamy O, et al. TBS (trabecular bone score) and diabetes-related fracture risk. *J Clin Endocrinol Metab.* 2013;98:602–609.

16. Dhaliwal R, Cibula D, Ghosh C, et al. Bone quality assessment in type 2 diabetes mellitus. *Osteoporos Int.* 2014;25:1969–1973.

17. Kim JH, Choi HJ, Ku EJ, et al. Trabecular bone score as an indicator for skeletal deterioration in diabetes. *J Clin Endocrinol Metab.* 2015;100:475–482.

18. Choi YJ, Ock SY, Chung YS. Trabecular Bone Score (TBS) and TBS-adjusted fracture risk assessment tool are potential supplementary tools for the discrimination of morphometric vertebral fractures in postmenopausal women with type 2 diabetes. *J Clin Densitom*. 2016;19:507–514.

19. Holloway KL, De Abreu LLF, Hans D, et al. Trabecular bone score in men and women with impaired fasting glucose and diabetes. *Calcif Tissue Int.* 2018;102:32–40.

20. Rianon N, Ambrose CG, Buni M, et al. Trabecular bone score is a valuable addition to bone mineral density for bone quality assessment in older Mexican American women with type 2 diabetes. *J Clin Densitom.* 2018;21:355–359.

21. Glüer CC. Quantitative ultrasound techniques for the assessment of osteoporosis: expert agreement on current status. The International Quantitative Ultrasound Consensus Group. *J Bone Miner Res.* 1997;12:1280–1288.

22. Patel S, Hyer S, Tweed K, et al. Risk factors for fractures and falls in older women with type 2 diabetes mellitus. *Calcif Tissue Int.* 2008;82:87–91.

23. Padilla F, Jenson F, Bousson V, et al. Relationships of trabecular bone structure with quantitative ultrasound parameters: In vitro study on human proximal femur using transmission and backscatter measurements. *Bone.* 2008;42:1193–1202.

24. Bouxsein ML, Radloff SE. Quantitative ultrasound of the calcaneus reflects the mechanical properties of calcaneal trabecular bone. *J Bone Miner Res.* 1997;12:839–846.

25. Patel S, Hyer S, Tweed K, et al. Risk factors for fractures and falls in older women with type 2 diabetes mellitus. *Calcif Tissue Int.* 2008;82:87–91.

26. Yamaguchi T, Yamamoto M, Kanazawa I, et al. Quantitative ultrasound and vertebral fractures in patients with type 2 diabetes. *J Bone Miner Metab.* 2011;29:626–632.

27. Tao B, Liu JM, Zhao HY, et al. Differences between measurements of bone mineral densities by quantitative ultrasound and dual-energy X-ray absorptiometry in type 2 diabetic postmenopausal women. *J Clin Endocrinol Metab.* 2008;93:1670–1675.

28. Bulló M, Garcia-Aloy M, Basora J, et al. Bone quantitative ultrasound measurements in relation to the metabolic syndrome and type 2 diabetes mellitus in a cohort of elderly subjects at high risk of cardiovascular disease from the PREDIMED study. *J Nutr Health Aging*. 2011;15:939–944.

29. Patsch JM, Burghardt AJ, Yap SP, et al. Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. *J Bone Miner Res.* 2013;28:313–324.

30. Samelson EJ, Demissie S, Cupples LA, et al. Diabetes and deficits in cortical bone density, microarchitecture, and bone size: Framingham HR-pQCT Study. *J Bone Miner Res.* 2018;33:54–62.

31. Burghardt AJ, Issever AS, Schwartz AV, et al. High-resolution peripheral quantitative computed tomographic imaging of cortical and trabecular bone microarchitecture in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab.* 2010;95:5045–5055.

32. Farr JN, Drake MT, Amin S, et al. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. *J Bone Miner Res.* 2014;29:787–795.

33. Paccou J, Ward KA, Jameson KA, et al. Bone microarchitecture in men and women with diabetes: the importance of cortical porosity. *Calcif Tissue Int.* 2016;98:465–473.

34. Nilsson A, Sundh D, Johansson L, et al. Type 2 diabetes mellitus is associated with better bone microarchitecture but lower bone material strength and poorer physical function in elderly women: a population-based study. *J Bone Miner Res.* 2017;32:1062–1071.

35. Arnett TR. Osteocytes: regulating the mineral reserves? *J Bone Miner Res.* 2013;28:2433–2435.

36. Kerschnitzki M, Kollmannsberger P, Burghammer M, et al. Architecture of the osteocyte network correlates with bone material quality. *J Bone Miner Res.* 2013;28:1837–1845.

37. Sinaki M, Pfeifer M, Preisinger E, et al. The role of exercise in the treatment of osteoporosis. *Curr Osteoporos Rep.* 2010;8:138–144.

38. Howe TE, Shea B, Dawson LJ, et al. Exercise for preventing and treating osteoporosis in postmenopausal women. *Cochrane Database Syst Rev.* 2011;(7):CD000333.

39. Karlsson M. Does exercise reduce the burden of fractures? A review. *Acta Orthop Scand.* 2002;73:691–705.

40. Sinaki M, Itoi E, Wahner HW, et al. Stronger back muscles reduce the incidence of vertebral fractures: a prospective 10 year follow-up of postmenopausal women. *Bone.* 2002;30:836–841.

41. Villareal DT, Chode S, Parimi N, et al. Weight loss, exercise, or both and physical function in obese older adults. *N Engl J Med.* 2011;364:1218–1229.

42. Shah K, Armamento-Villareal R, Parimi N, et al. Exercise training in obese older adults prevents increase in bone turnover and attenuates decrease in hip bone mineral density induced by weight loss despite decline in bone-active hormones. *J Bone Miner Res.* 2011;26:2851–2859

43. Villareal DT, Aguirre L, Gurney AB, et al. Aerobic or resistance exercise, or both, in dieting obese older adults. *N Engl J Med.* 2017;376:1943–1955.

44. Gillespie LD, Gillespie WJ, Robertson MC, et al. Interventions for preventing falls in elderly people. *Cochrane Database Syst Rev.* 2003;(4):CD000340.

45. Orlando G, Balducci S, Bazzucchi I, et al. Neuromuscular dysfunction in type 2 diabetes: underlying mechanisms and effect of resistance training. *Diabetes Metab Res Rev.* 2016;32:40–50.

46. American Diabetes Association. Standards of Medical Care in Diabetes—2018. *Diabetes Care.* 2018;41(Suppl. 1):S1–S159.

47. Inoue M, Iso H, Yamamoto S, et al. Daily total physical activity level and premature death in men and women: results from a large-scale population-based cohort study in Japan (JPHC study). *Ann Epidemiol.* 2008;18:522–530.

48. Sedentary Behaviour Research Network. Letter to the Editor: Standardized use of the terms "sedentary" and "sedentary behaviours". *Appl Physiol Nutr Metab.* 2012;37:540–542.

49. Balducci S; Zanuso S; Nicolucci A; et al. Effect of an intensive exercise intervention strategy on modifiable cardiovascular risk factors in type 2 diabetic subjects. A Randomized Controlled Trial: The Italian Diabetes and Exercise Study (IDES). *Arch Intern Med.* 2010;170:1794–1803.

50. Karlamangla AS, Barrett-Connor E, Young J, et al. Hip fracture risk assessment using composite indices of femoral neck strength: the Rancho Bernardo study. *Osteoporos Int.* 2004;15:62–70.

51. Russo CR, Lauretani F, Seeman E, et al. Structural adaptations to bone loss in aging men and women. *Bone.* 2006;38:112–118.

52. Balducci S, Sacchetti M, Orlando G, et al. Correlates of muscle strength in diabetes. The Study on the Assessment of Determinants of Muscle and Bone Strength Abnormalities in Diabetes (SAMBA). *Nutr Metab Cardiovasc Dis.* 2014;24:18–26.

53. Erlandson MC, Lorbergs AL, Mathur S, et al. Muscle analysis using pQCT, DXA and MRI. *Eur J Radiol.* 2016;85:1505–1511.

54. Balducci S, Zanuso S, Massarini M, et al. The Italian Diabetes and Exercise Study (IDES): Design and methods for a prospective Italian multicentre trial of intensive lifestyle intervention in people with type 2 diabetic and the metabolic Syndrome. *Nutr Metab Cardiovasc Dis.* 2008;18:585–595.

55. Guralnik JM, Ferrucci L, Simonsick EM, et al. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med.* 1995;332:556–561.

56. Talbot LA, Musiol RJ, Witham EK, et al. Falls in young, middle-aged and older community dwelling adults: perceived cause, environmental factors and injury. *BMC Public Health.* 2005;18;5:86.

57. Washburn RA, Smith KW, Jette AM, et al. The physical activity scale for the elderly (PASE): Development and evaluation. *J Clin Epidemiol.* 1993;46:153–162.

58. Balducci S, Vulpiani MC, Pugliese L, et al. Effect of supervised exercise training on musculoskeletal symptoms and function in patients with type 2 diabetes: the Italian Diabetes Exercise Study (IDES). *Acta Diabetol.* 2014;51:647–654.

59. Stevens RJ, Kothari V, Adler AI, et al. The UKPDS risk engine: a model for the risk of coronary heart disease in Type II diabetes (UKPDS 56). *Clin Sci (Lond).* 2001;101:671–679.

60. Singer JD, Willett JB. Applied longitudinal data analysis: modeling change and event occurrence. New York, NY, USA: Oxford University Press 2003.

61. Dufour R, Winzenrieth R, Heraud A, , et al. Generation and validation of a normative, age-specific reference curve for lumbar spine trabecular bone score (TBS) in French women. *Osteoporos Int.* 2013;24:2837-2846.

62. Iki M, Fujita Y, Tamaki J, et al. Trabecular bone score may improve FRAX® prediction accuracy for major osteoporotic fractures in elderly Japanese men: the Fujiwara-kyo Osteoporosis Risk in Men (FORMEN) Cohort Study. *Osteoporos Int.* 2015;26:1841-1848.

63. Krieg MA, Aubry-Rozier B, Hans D, et al. Effects of anti-resorptive agents on trabecular bone score (TBS) in older women. *Osteoporos Int.* 2013;24:1073-1078.

64. Leslie WD, Majumdar SR, Morin SN, et al. Change in Trabecular Bone Score (TBS) With Antiresorptive Therapy Does Not Predict Fracture in Women: The Manitoba BMD Cohort. *J Bone Miner Res.* 2017;32:618-623.

65. Ho-Pham LT, Nguyen TV. Association between trabecular bone score and type 2 diabetes: a quantitative update of evidence. *Osteoporos Int.* 2019 Jun 18. doi: 10.1007/s00198-019-05053-z.

66. Senn C, Günther B, Popp AW, et al. Comparative effects of teriparatide and ibandronate on spine bone mineral density (BMD) and microarchitecture (TBS) in postmenopausal women with osteoporosis: a 2-year open-label study. *Osteoporos Int.* 2014;25:1945–1951.

67. Di Gregorio S, Del Rio L, Rodriguez-Tolra J, et al. Comparison between different bone treatments on areal bone mineral density (aBMD) and bone microarchitectural texture as assessed by the trabecular bone score (TBS). *Bone.* 2015;75:138–143.

68. Saag KG, Agnusdei D, Hans D, et al. Trabecular bone score in patients with chronic glucocorticoid therapy-induced osteoporosis treated with alendronate or teriparatide. *Arthritis Rheumatol.* 2016;68:2122–2128.

69. Russo CR. The effects of exercise on bone. Basic concepts and implications for the prevention of fractures. *Clin Cases Miner Bone Metab.* 2009;6:223-228.

70. Jain RK, Vokes T. Physical activity as measured by accelerometer in NHANES 2005-2006 is associated with better bone density and trabecular bone score in older adults. *Arch Osteoporos.* 2019;14:29.

71. International Committee of Medical Journal Editors. Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. 2017, (accessed 18 October, 2018).

**Authors' contributions:** SB, FGC and GP conceived and designed the study. All the other authors made substantial contributions to specific parts of the protocol: recruitment and follow-up program (MV and LB), training program (MS, GO, GR, and SZ), imaging procedures (GA, LP, and AL), QUS and pQCT protocols (CRR, JH, and VDE), biochemical testing panel (PC), and statistical analysis plan (AN). GP drafted the manuscript; SB, FGC, MS, CRR, GA, JH, GO, GR, VDE, PC, LP, AL, MV, LB, SZ, and AN revised the manuscript critically for important intellectual content. All authors have given their final approval of the manuscript to be published.

**Funding statement:** This work is supported by the Metabolic Fitness Association, Monterotondo, Rome, Italy, by the EFSD/Novo Nordisk A/S Programme for Diabetes Research in Europe 2019 to GP, and, for the pilot study, by a grant from the Italian Ministry of Health (RF-2010-2310812) to GP. The funding sources have no role in design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the report for publication.

**Declaration of interest:** The authors declare no competing interests.

**Data sharing statement:** The data generated by this trial will be made available from the corresponding author on reasonable request.

## Figure Legends

**Figure 1.** Study flow chart. LTPA = leisure-time physical activity; PASE = Physical Activity Scale for the Elderly; MS = musculoskeletal; DXA = dual-energy X-ray absorptiometry; QUS = quantitative ultrasound; pQCT = peripheral quantitative computed tomography.

**Figure 2.** Sequence of exercises during each supervised exercise training session. * Intensity of aerobic exercise will be adjusted according to improvements in predicted $VO_{2max}$, as recorded every 6 months. † Intensity of resistance exercise will be adjusted according to improvements in 1-RM, as recorded every 6 months; new resistance exercises will be introduced every 12 weeks to maintain patient's adherence, and the velocity of execution during the concentric phase of the movement will be progressively increased to enhance muscle power. ‡ Height of jumps and amplitude of movements of weight bearing exercise will be also progressively increased. § Difficulty level of balance training will be gradually increased by performing the exercises with closed eyes, reducing the support area, changing visual fixation (e.g., head rotations), varying the centre of mass (e.g., limb raising), or adding a manual or cognitive task. $VO_{2max}$ = maximal oxygen consumption; 1-RM = one-repetition maximum.

**Table 1.** Exclusion criteria.

- Unable or unwilling to give informed consent or communicate with local study staff
- Current diagnosis of psychiatric disorder or hospitalization for depression in the past six months
- Self-reported alcohol or substance abuse within the past twelve months
- Self-reported inability to walk two blocks
- Musculoskeletal disorders or deformities that may interfere with participation in the intervention
- History of central nervous dysfunction such as hemiparesis, myelopathies, cerebral ataxia
- Clinical evidence of vestibular dysfunction
- Postural hypotension defined as a fall in BP when changing position of >20 mmHg (systole) or >10 mmHg (diastole)
- Cancer requiring treatment in the past five years, except for cancers that have clearly been cured or in the opinion of the investigator carry an excellent prognosis (e.g., stage 1 cervical cancer)
- Chronic obstructive pulmonary disease
- End-stage liver disease
- Chronic diabetic complications:
  - recent major acute cardiovascular event, including heart attack, stroke/transient ischemic attack(s), revascularization procedure, or participation in a cardiac rehabilitation program within the past three months
  - pre-proliferative and proliferative retinopathy
  - macroalbuminuria and/or eGFR < 45 ml/min/1.73 $m^2$
  - severe motor and sensory neuropathy
  - diabetic foot with history of ulcer
- Cardiovascular disease at cardiologic examination:
  - history of cardiac arrest
  - history of pulmonary embolism in the past six months
  - unstable angina pectoris or angina pectoris at rest
  - resting HR <45 beats/min or >100 beats/min
  - complex ventricular arrhythmia at rest or with exercise
  - uncontrolled atrial fibrillation (HR $\geq$100 beats/min)
  - NYHA Class III or IV congestive heart failure
  - acute myocarditis, pericarditis or hypertrophic myocardiopathy
  - left bundle branch block or cardiac pacemaker

- ECG treadmill test suggestive of myocardial ischemia

- Poor glycaemic and blood pressure (BP) control
  - haemoglobin (Hb) $A_{1c}$ >9.0%
  - BP >150/90 mmHg
- Bone abnormalities
  - vitamin D <10 ng/ml
  - treatment with anti-fracturative agents, estrogens, aromatase inhibitors, testosterone, corticosteroids and/or glitazones
  - previous documented non-traumatic fractures
  - SDI >5 (and >2 in a single vertebra)
  - T score ≤-2.5 at spine/hip at DXA
- Conditions not specifically mentioned above at the discretion of the clinical site

---

BP = blood pressure; eGFR = estimated glomerular filtration rate; HR = heart rate; NYHA = New York Heart Association; ECG = electrocardiogram; SDI = total spine deformity index; DXA = dual-energy X-ray absorptiometry. Participants with $HbA_{1c}$ or BP above the indicated threshold will be receive appropriate treatment and will be re-evaluated after 3 months. Patients with vitamin D levels < 10 ng/dl will be treated with cholecalciferol 25.000 IU/week for 6 weeks and will be re-evaluated 2 weeks after the last dose.

**Table 2.** Methods for measurements of markers of bone turnover.

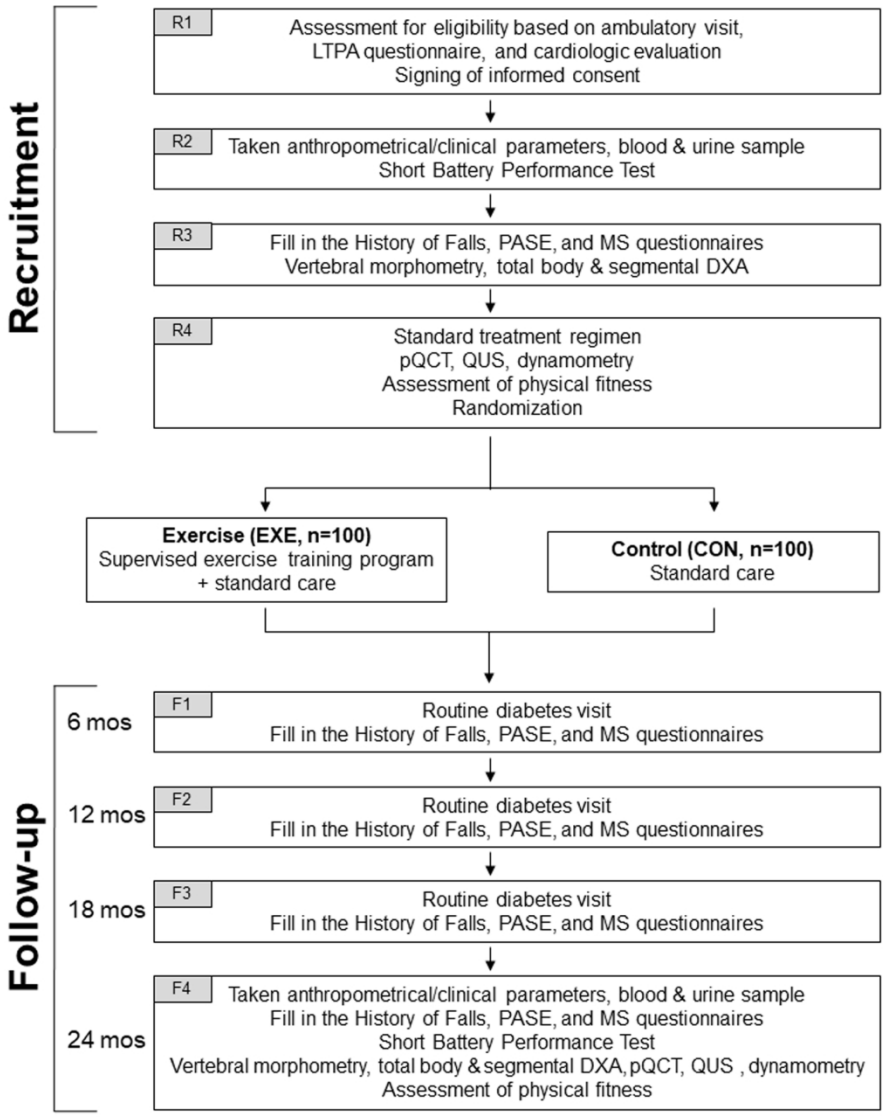
| Analyte | Method | Manifacturer |
|---|---|---|
| **Ca** | Colorimetric spectrophotometric | Architect, Abbot Diagnostics, Lake Forest, IL, USA |
| **P** | Colorimetric spectrophotometric | Architect, Abbot Diagnostics, Lake Forest, IL, USA |
| **25OH Vitamin D** | Competitive ECLIA | Liaison, DiaSorin SpA, Saluggia, Italy |
| **PTH** | ECLIA | Liaison, DiaSorin SpA, Saluggia, Italy |
| **Total ALP** | Colorimetric spectrophotometric | Architect, Abbot Diagnostics, Lake Forest, IL, USA |
| **Bone-specific ALP** | ECLIA | Liaison, DiaSorin SpA, Saluggia, Italy |
| **Osteocalcin** | ELISA | RayBiotech, Norcross, GA, USA |
| **PINP** | ELISA | RayBiotech, Norcross, GA, USA |
| **CTX-1** | ELISA | RayBiotech, Norcross, GA, USA |
| **TRAcP 5b** | ELISA | RayBiotech, Norcross, GA, USA |
| **Sclerostin** | ELISA | RayBiotech, Norcross, GA, USA |
| **DKK-1** | ELISA | RayBiotech, Norcross, GA, USA |

Ca = calcium; P = phosphorus; PTH = parathyroid hormone; ALP = alkaline phosphatase; PINP = procollagen I intact N-terminal; CTX-1 = C-terminal telopeptide of type I collagen; TRAcP 5b = tartrate-resistant acid phosphatase 5b; DKK-1= Dickkopf-1; ECLIA = chemiluminescent immunoassay; ELISA; enzyme-linked immunosorbent assay.

**Table 3.** Methods for measurements of cardiovascular risk factors.

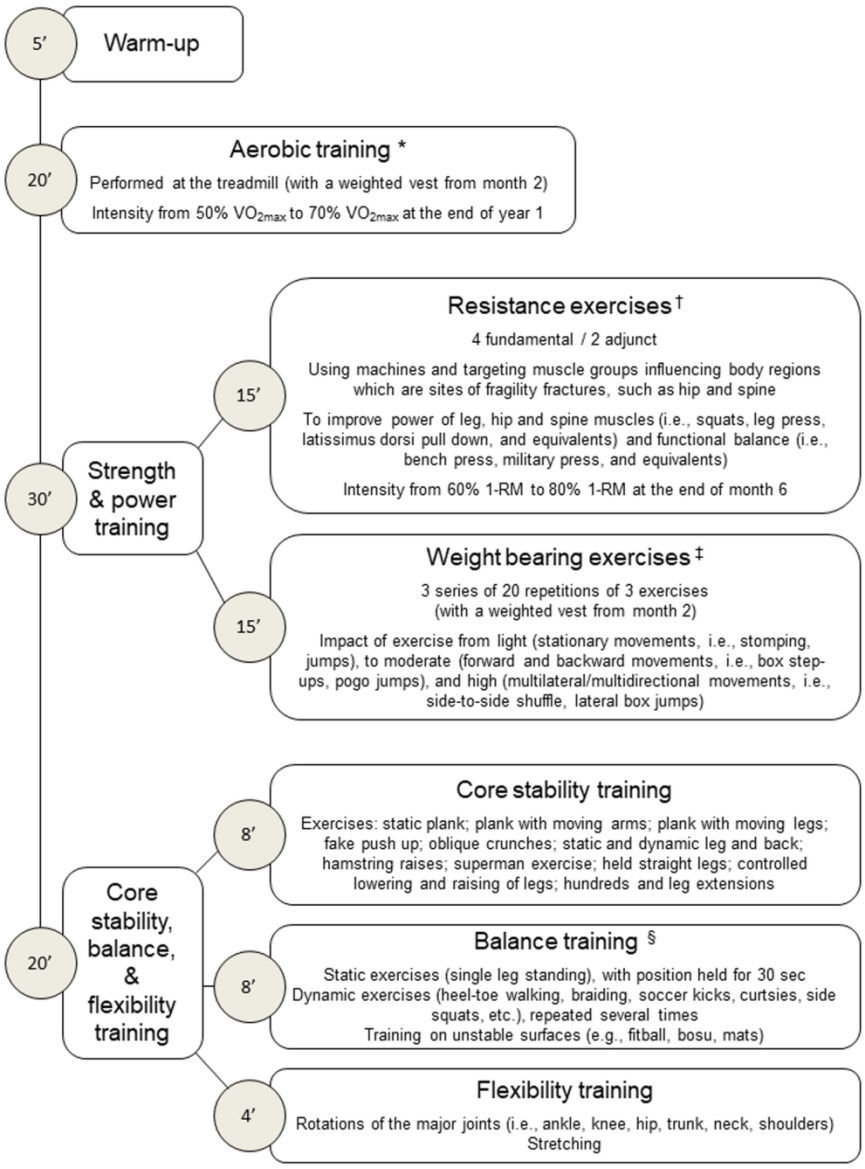
| Analyte | Method | Manifacturer |
|---|---|---|
| **$HbA_{1c}$** | HPLC (Adams TMA1C HA-8160) | Menarini Diagnostics, Florence, Italy |
| **FPG** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Triglycerides** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Total cholesterol** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **HDL cholesterol** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **hs-CRP** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Blood count** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Uric acid** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Serum creatinine** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Urinary albumin** | mAlb VITROS | Ortho Clinical Diagnostics Inc, Raritan, NJ, USA |
| **Urinary creatinine** | VITROS 5,1 FS Chemistry System | Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA |

$HbA_{1c}$ = haemoglobin $A_{1c}$; HPLC = high-performance liquid chromatography; FPG = fasting plasma glucose; hs-CRP= high sensitivity-C-reactive protein. LDL cholesterol will be calculated using the Friedewald formula (https://www.mdcalc.com/ldl-calculated), whereas glomerular filtration rate (GFR) will be estimated from serum creatinine by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (http://www.qxmd.com/calculate-online/nephrology/ckd-epi-egfr).




Study flow chart. LTPA = leisure-time physical activity; PASE = Physical Activity Scale for the Elderly; MS = musculoskeletal; DXA = dual-energy X-ray absorptiometry; QUS = quantitative ultrasound; pQCT = peripheral quantitative computed tomography.

142x189mm (300 x 300 DPI)




Sequence of exercises during each supervised exercise training session. * Intensity of aerobic exercise will be adjusted according to improvements in predicted VO2max, as recorded every 6 months. † Intensity of resistance exercise will be adjusted according to improvements in 1-RM, as recorded every 6 months; new resistance exercises will be introduced every 12 weeks to maintain patient's adherence, and the velocity of execution during the concentric phase of the movement will be progressively increased to enhance muscle power. ‡ Height of jumps and amplitude of movements of weight bearing exercise will be also progressively increased. § Difficulty level of balance training will be gradually increased by performing the exercises with closed eyes, reducing the support area, changing visual fixation (e.g., head rotations), varying the centre of mass (e.g., limb raising), or adding a manual or cognitive task. $VO_{2max}$ = maximal oxygen consumption; 1-RM = one-repetition maximum.

142x189mm (300 x 300 DPI)

## Appendix A: List of participants

**Recruitment and follow-up:** Diabetes Unit, Sant'Andrea Hospital, Rome, Italy: Giuseppe Pugliese, Stefano Balducci, Martina Vitale, Tiziana Cirrito, Lucilla Bollanti, Francesco G. Conti.

**Supervised exercise training:** Metabolic Fitness Association, Monterotondo, Rome, Italy: Stefano Balducci, Gianluca Balducci, Enza Spinelli.

**DXA and vertebral morphometry evaluation:** Radiology Unit, Sant'Andrea University Hospital, Rome, Italy Giuseppe Argento, Luca Pugliese, Andrea Laghi.

**QUS and pQCT evaluation:** Metagym Fitness Centre, Florence, Italy: Cosimo R. Russo; Diabetes Unit, Sant'Andrea Hospital, Rome, Italy: Jonida Haxhi, Valeria D'Errico.

**Physical Fitness evaluation:** Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy: Massimo Sacchetti, Giorgio Orlando, Olimpia Andreani; Diabetes Unit, Sant'Andrea Hospital, Rome, Italy: Gianvito Rapisarda, Eugenio Santacroce.

**Questionnaire evaluation:** Centre for Applied Biological & Exercise Sciences, Faculty of Health & Life Sciences, Coventry University, Coventry, UK: Silvano Zanuso.

**Laboratory testing:** Laboratory of Clinical Chemistry, Sant'Andrea Hospital, Rome, Italy: Patrizia Cardelli, Gerardo Salerno, Stefano Cavallo.

**Statistical Analysis:** Centre for Outcomes Research and Clinical Epidemiology (CORE), Pescara, Italy: Antonio Nicolucci, Giuseppe Lucisano.

**Steering Committee:** Giuseppe Pugliese, Stefano Balducci, Francesco G. Conti, Massimo Sacchetti, Cosimo R, Russo, Giuseppe Argento, Silvano Zanuso, Patrizia Cardelli, Antonio Nicolucci.

# Appendix B: Questionnaires

**B1: History of Falls questionnaire**

**B2: Physical Activity Scale for the Elderly (PASE) questionnaire**

**B3: Self-report questionnaire for musculoskeletal (MS) symptoms**

## B1: History of Falls questionnaire

| | |
|---|---|
| **A. Activities prior to falling** | |
| 1. Ambulation | |
| 2. Transferring | |
| 3. Running | |
| 4. Sports | |
| 5. Stairs/curb | |
| 6. Other | |
| **B. Perceived causes (accident/environmental-related)** | |
| 1. Collapse episode | |
| 2. Dizziness/vertigo | |
| 3. Balance/gait impairment | |
| 4. Other | |
| **C. Perceived causes (environmental factors)** | |
| 1. Wet surface | |
| 2. Uneven surface/steps | |
| 3. Objects on surface/rugs | |
| 4. External forces | |
| 5. Icy surfaces | |
| 6. Other | |
| **D. Injuries sustained from fall** | |
| 1. Fractures | |
| 2. Treated injury | |
| 3. Untreated injury | |
| 4. No injury | |

## B2: Physical Activity Scale for the Elderly (PASE) questionnaire

**Q1.** Over the past 7 days, how often did you participate in sitting activities such as reading, watching TV, or doing handcrafts?

**Q1b.** On average, how many hours per day did you engage in these sitting activities?

**Q2.** Over the past 7 days, how often did you take a walk outside your home or yard for any reason? For example, for fun or exercise, walking to work, walking the dog, walking in a mall, etc?

**Q2a.** On average, how many hours per day did you spend walking?

**Q3.** Over the past 7 days, how often did you engage in light sport or recreational activities such as bowling, golf with a cart, shuffleboard, fishing from a boat or pier or other similar activities?

**Q3b.** On average, how many hours per day did you engage in these light sport or recreational activities?

**Q4.** Over the past 7 days, how often did you engage in moderate sport or recreational activities such as doubles tennis, ballroom dancing, hunting, ice skating, golf without a cart, softball or other similar activities?

**Q4b.** On average, how many hours per day did you engage in these moderate sport or recreational activities?

**Q5.** Over the past 7 days, how often did you engage in strenuous sport or recreational activities such as jogging, swimming, cycling, singles tennis, aerobic dance, skiing (downhill or cross country or other similar activities?

**Q5b.** On average, how many hours per day did you engage in these strenuous activities?

**Q6.** Over the past 7 days, how often did you do any exercises specifically to increase muscle strength or endurance, such as lifting weights or pushups, etc?

**Q6a.** On average, how many hours per day did you engage in exercises to increase muscle strength or endurance, such as lifting weights, pushups, or physical therapy with weights, etc.?

**Q7.** During the past 7 days, have you done any light housework, such as dusting, washing or drying dishes, or ironing?

**Q8.** During the past 7 days, have you done any heavy housework or chores such as vacuuming, scrubbing floors, washing windows, or carrying wood?

**Q9a.** During the past 7 days, did you engage in home repairs like painting, wallpapering, electrical work, etc.?

**Q9b.** During the past 7 days, did you engage in lawn work or yard care, including snow or leaf removal, chopping wood, etc?

**Q9c.** During the past 7 days, did you engage in outdoor gardening?

**Q9d.** During the past 7 days, did you engage in caring for another person such as a child, dependent spouse, or another adult?

**Q10.** During the past 7 days, did you work for pay or as a volunteer?

**Q10a.** How many hours per week did you work for pay and/or as a volunteer?

**Q10b.** Which of the following categories best describes the amount of physical activity required on your job and/or volunteer work?

Category 1 ("Mainly sitting with slight arm movements") includes examples such as: office worker, watchmaker, seated assembly line worker, bus driver, etc.

Category 2 ("Sitting or standing with some walking") includes examples such as: cashier, general office worker, light tool and machinery worker.

Category 3 ("Walking, with some handling of materials generally weighing less than 50 pounds") includes examples such as: mailman, waiter/waitress, construction worker, heavy tool and machinery worker.

Category 4 ("Walking and heavy manual work often requiring handling of materials weighing over 50 pounds") includes examples such as: lumberjack, stonemason, farm or general labourer].

## B3. Self-reported questionnaire for MS symptoms

| | |
|---|---|
| **SHOULDER** | |
| **1** | Do you have pain during rotation of the arm? |
| **2** | Are you awakened by pain during the night? |
| **3** | Do you have pain on reaching objects above the head? |
| **4** | Do you have pain on lifting objects? |
| **5** | Do you have pain or soreness upon awakening that passes later on during the day? |
| **6** | Have you taken anti-inflammation drugs or pain-killers? |
| **ARM** | |
| **7** | Do you feel that you have less strength? |
| **8** | Dou you feel that one arm is weaker than the other? |
| **9** | Do you have pain at the maximum extension of the forearm? |
| **ELBOW** | |
| **10** | Do you have pain on lifting an object? |
| **11** | Do you have pain on hitting against a rigid object? |
| **12** | Have you taken anti-inflammation drugs or pain-killers? |
| **WRIST** | |
| **13** | Do you have pain on lifting an object? |
| **14** | Do you have pain on hitting against a rigid object? |
| **15** | Have you taken anti-inflammation drugs or pain-killers? |
| **HAND:** Do you feel "pins and needles"? If so, in which finger? | |
| **16** | I |
| **17** | II |
| **18** | III |
| **19** | IV |
| **20** | V |
| **SPINE: THORACO-CERVICAL** | |
| **21** | Do you have pain/tenderness/ pins and needles on turning your head from side to side? |
| **22** | Do you often have pain or headache or heaviness of the head or neck? |
| **23** | Do you have pain between the shoulder blades? |
| **24** | Do you feel it necessary to move your head from side to side to get moving and feel? |
| **25** | Do you have episodes of painful sudden acute stiffness of the neck? |
| **26** | Have you taken anti-inflammation drugs or pain-killers? |
| **SPINE: LUMBO-SACRAL** | |
| **27** | Do you have pain on bending to tie your shoe laces? |
| **28** | Do you have any back-pain on turning left or right? |
| **29** | Do you have a feeling of heaviness in your back on standing for long hours? |
| **30** | Do you have bothersome feeling when sitting still? Do you have to get up? |
| **31** | Did you have one episode of sudden intense back pain that leaves you unable to move? |
| **32** | Have you taken anti-inflammation drugs or pain-killers? |

| | |
|---|---|
| **HIP** | |
| **33** | Do you have pain on crossing your legs? |
| **34** | Do you have any pain when opening your legs to the maximum? |
| **35** | Do you often have pain from your buttocks along the length of the leg down to your ankles? |
| **36** | Have you taken a single dose of anti-inflammatory drugs or pain-killers? |
| **KNEE** | |
| **37** | Do you have pain in the knee in the act of sitting down or getting up? |
| **38** | Do you have pain in your knee after having walked a lot? |
| **39** | Is your knee often swollen at the end of the day? |
| **40** | Do you have pain in the "good" knee? |
| **41** | Do you have pain or a bothersome feeling as you kneel down? |
| **42** | When lying in bed, do you feel the need to move your legs, ones or more than once? |
| **43** | Have you taken a single dose of anti-inflammatory drugs or pain-killers? |
| **FOOT** | |
| **44** | Do you often feel a sensation of pins and needles that runs down to one or more toes? |
| **45** | Do you have any difficulty in standing on your toes? |
| **46** | Do you have any pain in your foot after walking for a long time? |
| **47** | Do you have pain on taking the first step in the morning? |
| **48** | Do you have any difficulty or pain when putting on stiff orthopaedic shoes? |
| **49** | Do you have pain under the heel when walking a lot? |
| **50** | Have you taken a single dose of anti-inflammatory drugs or pain-killers? |

## Appendix C: World Health Organization Trial Registration Data Set

1. **Primary Registry and Trial Identifying Number:** ClinicalTrials.gov; NCT02421393; URL https://clinicaltrials.gov/ct2/show/NCT02421393.
2. **Date of Registration in Primary Registry:** 20 April. 2014
3. **Secondary Identifying Numbers:** NA.
4. **Source(s) of Monetary or Material Support:** Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: info@metabolicfitness.it.
5. **Primary Sponsor:** Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: info@metabolicfitness.it.
6. **Secondary Sponsor(s):** European Foundation for the Study of Diabetes, Rheindorfer Weg 3 - 40591 Düsseldorf, Germany; Phone: +49 211 758469 0; Fax: +49 211 758 469 29; E-mail: foundation@easd.org.
7. **Contact for Public Queries: Stefano Balducci, MD,** Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: sbalducci@esinet.it.
8. **Contact for Scientific Queries: Giuseppe Pugliese,** M.D., Ph.D., Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, Via di Grottarossa, 1035-1039 - 00189 Rome, Italy; Phone: +39-0633775440; Fax: +39-0633776327; E-mail: giuseppe.pugliese@uniroma1.it.
9. **Public Title:** The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes.
10. **Scientific Title:** The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes.
11. **Countries of Recruitment:** Italy.
12. **Health Condition(s) or Problem(s) Studied:** type 2 diabetes (T2D).
13. **Intervention:**
    a. Intervention arm
       - Name: Supervised exercise training.
       - Description: two weekly supervised mixed exercise training sessions for two years, on top of standard care.
    b. Standard care.
14. **Key Inclusion and Exclusion Criteria**
    a. Inclusion criteria: known T2D (defined by the ADA criteria) of at least 1-year duration. Additional requirements are age 40-80 years; BMI 27-40 kg/m$^2$; sedentary lifestyle (i.e., more than 8 hours/day spent in any waking behaviour characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture) and physically inactivity (i.e., insufficient amounts of PA according to current guidelines) from at least 6 months; a Short Battery Performance Test score ≥4; ability to walk 1.6 Km without assistance; and eligibility after cardiologic evaluation.

b. Exclusion criteria: unable or unwilling to give informed consent or communicate with local study staff; current diagnosis of psychiatric disorder or hospitalization for depression in the past six months; self-reported alcohol or substance abuse within the past twelve months; self-reported inability to walk two blocks; musculoskeletal disorders or deformities that may interfere with participation in the intervention; history of central nervous dysfunction such as hemiparesis; myelopathies; cerebral ataxia; clinical evidence of vestibular dysfunction; postural hypotension defined as a fall in BP when changing position of >20 mmHg (systole) or >10 mmHg (diastole); currently pregnant or nursing; cancer requiring treatment in the past five years, except for cancers that have clearly been cured or in the opinion of the investigator carry an excellent prognosis (e.g., stage 1 cervical cancer); chronic obstructive pulmonary disease; end-stage liver disease; chronic diabetic complications (recent major acute cardiovascular event, including heart attack, stroke/transient ischemic attack(s), revascularization procedure, or participation in a cardiac rehabilitation program within the past three months; pre-proliferative and proliferative retinopathy; macroalbuminuria and/or eGFR < 45 ml/min/1.73 $m^2$; severe motor and sensory neuropathy; diabetic foot with history of ulcer); cardiovascular disease at cardiologic examination (history of cardiac arrest; history of pulmonary embolism in the past six months; unstable angina pectoris or angina pectoris at rest; resting HR <45 beats/min or >100 beats/min; complex ventricular arrhythmia at rest or with exercise; uncontrolled atrial fibrillation with HR >100 beats/min; NYHA Class III or IV congestive heart failure; acute myocarditis; pericarditis or hypertrophic myocardiopathy; left bundle branch block or cardiac pacemaker); treatment with anti-fracture agents, oestrogens, aromatase inhibitors, testosterone, corticosteroids and/or glitazones; previous documented non-traumatic fractures; spinal deformity index (SDI) >3 (and >2 in a single vertebra); and a T score <-2.5 at spine/hip at DXA; haemoglobin (Hb) $A_{1c}$ >9.0%; blood pressure (BP) >150/90 mmHg; vitamin D <10 ng/ml; conditions not specifically mentioned above at the discretion of the clinical site.

15. **Study Type**

a. Type of study: interventional.

b. Study design:

- Method of allocation: randomized
- Masking: no (assessor-blinded)
- Assignment: parallel
- Purpose: testing the efficacy of a specific exercise training program in improving bone quality and strength in patients with T2D

c. Phase: NA

d. Allocation concealment mechanism and sequence generation: centralized randomization stratified by age, gender, and type of diabetes treatment (non-insulin versus insulin therapy), using a permuted-block randomization software which randomly varies the block size.

15. **Date of First Enrolment:** November 1, 2018 (expected).

16. **Target Sample Size:** 200

17. **Recruitment Status: recruiting.**

16. **Primary Outcome(s)**

- Name: baseline to end-of-study change in Trabecular Bone Score (TBS);
- Method of measurement: spine dual-energy X-ray absorptiometry (DXA)-derived software-based measure;

- Time points: baseline and end-of-study.

17. **Key Secondary Outcomes**

a. Name: baseline to end-of-study change in broadband ultrasound attenuation (BUA), speed of sound (SOS), and quantitative ultrasound index (QUI); methods of measurement: quantitative ultrasound (QUS); time points: baseline and end-of-study.

b. Name: baseline to end-of-study change in multiple measured and calculated bone parameters; methods of measurement: peripheral quantitative computed tomography (pQCT); time points: baseline and end-of-study.

c. Name: baseline to end-of-study change in bone mineral density (BMD) and other DEXA-derived measures; method of measurement: spine and hip DXA; time points: baseline and end-of-study.

d. Name: baseline to end-of-study change in markers of bone turnover; method of measurement: immunochemical methods; time points: baseline and end-of-study.

e. Name: baseline to end-of-study change in body composition; method of measurement: total body DXA; time points: baseline and end-of-study.

f. Name: baseline to end-of-study change in muscle strength; methods of measurement: isometric muscle strength test; time points: baseline and end-of-study.

g. Name: baseline to end-of-study change in muscle cross-sectional area; method of measurement: pQCT; time points: baseline and end-of-study.

h. Name: baseline to end-of-study change in balance, gait and power; method of measurement: Short Battery Performance Test; time points: baseline and end-of-study.

i. Name: number of falls; 17-item History of Falls questionnaire; time points: baseline and every 6 months thereafter for 7 years (2-year trial + 5-year post-trial follow-up).

j. Name: symptomatic fractures; method of measurement: clinical and radiographic records; time points: baseline and every 6 months thereafter for 7 years (2-year trial + 5-year post-trial follow-up).

k. Name: asymptomatic and symptomatic fractures; method of measurement: vertebral morphometry; time points: baseline and every 6 months thereafter for 7 years (2-year trial + 5-year post-trial follow-up).

## Appendix D: Informed consent

### Patient Information Sheet

### TITLE OF THE STUDY: The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes

This study is registered at ClinicalTrial.gov as "Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET-BONE) in type 2 diabetes: an exercise intervention program for reducing the risk of fractures "(N. NCT02421393, URL https://clinicaltrials.gov/ct2/show/NCT02421393)

**IDENTIFICATION OF THE STUDY**

Dear Sir/Madam,

The study, which your physician (diabetes specialist) is inviting you to participate in, aims to evaluate the effect of 2-year training consisting of supervised and combined aerobic and resistance exercise sessions in individuals with type 2 diabetes mellitus on:

- bone quality and mass;
- bone metabolism;
- muscle strength and mass;
- balance and gait;
- falls;
- symptomatic and asymptomatic fractures.

The hypothesis is that a specific exercise training program produces a significant improvement in the qualitative and quantitative bone parameters by influencing bone metabolism, with a consequent reduction in the risk of fractures and, in the long term, a significantly reduced number of fractures.

The research involves about 200 patients with type 2 diabetes.

During the study you will be assigned to one of the following two groups:

1. Exercise (EXE) group, which receives standard care and participates in two weekly mixed exercise training sessions for two years, supervised by an exercise specialist at the Metabolic Fitness Association.
2. Control (CON) group, which receives only standard care.

The study will have a duration of 2 years plus a 5-year post-trial follow-up, during which you will be subjected to 6-month monitoring visits. The parameters reported above will be assessed at the beginning and at the end of the 2-year period, except for falls and fractures, which will be assessed every 6 months for the entire 7 year period (2-year trial + 5-year post-trial follow-up).

**PROTECTION OF PERSONAL DATA:**

- All information concerning you, the collection and processing of which is connected and indispensable to the achievement of the objectives of this study, will be treated in a manner suitable to ensure

absolute confidentiality and security in accordance with the provisions for the protection of personal data and the right to privacy (Italian Data Protection Act, No. 675 of December 6, 1996 and subsequent amendments/additions).

- You will be identified by a code and the clinical information concerning you will not be disclosed without your written permission. The data collected will consist of your initials, date of birth, sex and otherwise sensitive clinical data as suitable to reveal your state of health.
- As a participant in the processing of your personal data, you will have full access, through your family doctor, to the information concerning you. You will also have the right to exercise all the rights of cancellation, transformation, integration, updating, correction and blocking of your data within the limits set out in art. 13 of the Italian Data Protection Act 675/96 mentioned above. You will not be charged any fee for the scheduled exams, the results of which will be promptly communicated to your family doctor.

**STUDY BENEFITS:**

- Upon agreeing to participate in this study, you might be assigned to follow a supervised exercise training program. Whatever group you are assigned to, you will be under strict control by a staff-member, medical or otherwise, specialized in the management of type 2 diabetes, including physical activity/exercise therapy.
- In addition, your doctor may become aware of the presence of cardiovascular risk factors or complications to be monitored.
- Finally, the knowledge acquired thanks to your participation will be useful both for you and for other patients.

**PARTICIPATION IN THE STUDY:**

- Your participation in this study is completely cost-free and, if you decide not to take part, you will still be assisted in the most appropriate medical treatment.
- We invite you to ask your family physician any question you deem appropriate. Your doctor will also ask you to sign and date the consent form for the processing of personal data to confirm that you have read all the information contained herein, which includes that you have understood the aims of the study and most importantly, that you have freely given your consent to the collection and processing of your personal data.

## Patient Consent Form

**TITLE OF THE STUDY: The Study to Weigh the Effect of Exercise Training on BONE quality and strength (SWEET BONE) in type 2 diabetes**

I, the undersigned _____ born in _____ on _____

and resident in _____

hereby declare, after reading the information, the following:

- to have read and understood the patient information sheet of the aforementioned study and to have had ample time and opportunity to ask questions and obtain satisfactory answers to the investigator;
- to have understood that my participation is voluntary and that I can withdraw from the study at any time, without having to explain or influence any future medical assistance in any way;
- to have understood that my personal data will be processed according to the regulations in force specified in the information sheet of the study and that I can exercise my rights by contacting the Data Controller at any time and in the manner specified in accordance with art. 7, Legislative Decree n. 196 of 30/06/2003, (so-called Privacy Code).

Following these statements, I declare that I, the undersigned, freely:

- accept to participate in the study mentioned above;
- consent to the processing of personal and sensitive data collected in the context of this study, in the terms and methods indicated and explained in the information, aware that anonymity in the treatment will be guaranteed;
- consent that the investigator and his collaborators, as expressly indicated in the informative report, collect and process the data deriving from the investigations for the express purpose of a scientific publication.

**Signature of the patient** _____ **date** _____

**Surname and name of the patient** _____

**Signature of the investigator** _____ **date** _____

**Surname and name of the investigator** _____

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


| Section/item | Item No | Description | Addressed on page number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 3, 16 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | Appendix C |
| Protocol version | 3 | Date and version identifier | 16 |
| Funding | 4 | Sources and types of financial, material, and other support | 25 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1, 25 |
| | 5b | Name and contact information for the trial sponsor | 25, Appendix C |
| | 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 | 25 |
| | 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) | Appendix A |
| **Introduction** | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 5-6 |

| | | | |
|---|---|---|---|
| | 6b | Explanation for choice of comparators | N/A |
| Objectives | 7 | Specific objectives or hypotheses | 6 |
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 7 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 8, Appendix A |
| 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) | 7, Table 1 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 10-11, Fig. 2 |
| | 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) | N/A |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | 8 |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 11 |
| 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 | 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) | 8-10, Fig. 1 |
| Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 14 |
| Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 8 |

**Methods: Assignment of interventions (for controlled trials)**

Allocation:

| | | | |
|---|---|---|---|
| Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 9 |
| Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 9 |
| Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 9 |
| Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 9, 11 |
| | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | N/A |

**Methods: Data collection, management, and analysis**

| | | | |
|---|---|---|---|
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 11-14, Tables 2-3, Appendix B |
| | 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 | 8 |
| 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 | 14 |

| | | | |
|---|---|---|---|
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 14, 15 |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 15 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | 15 |
| **Methods: Monitoring** | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | N/A |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | N/A |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 14 |
| Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | N/A |
| **Ethics and dissemination** | | | |
| Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 16 |
| 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) | 16 |
| Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 8 |
| | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | N/A |

| | | | |
|---|---|---|---|
| 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 | 14 |
| Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 25 |
| Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 14 |
| 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 | 14 |
| Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 16 |
| | 31b | Authorship eligibility guidelines and any intended use of professional writers | 16 |
| | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 16, 25 |
| **Appendices** | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Appendix D |
| 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 | N/A |

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