The Italian Diabetes and Exercise Study 2 (IDES_2) Authors

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Participating centers

Diabetes Clinics

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2. Diabetes Unit, Fatebenefratelli San Pietro Hospital, Rome, Italy: Nicolina Di Biase, Filomena La Saracina.
3. Diabetes Unit, Health District, Monterotondo, Rome, Italy: Stefano Balducci, Mario Ranuzzi, Jonida Haxhi, Valeria D'Errico.

Metabolic Fitness Centers

1. Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy: Massimo Sacchetti, Giorgio Orlando.
2. Center for the Study of Metabolism, Rome, Italy: Luca Milo, Roberto Milo.
3. Metabolic Fitness Association, Monterotondo, Rome, Italy: Gianluca Balducci, Enza Spinelli.

Central laboratory

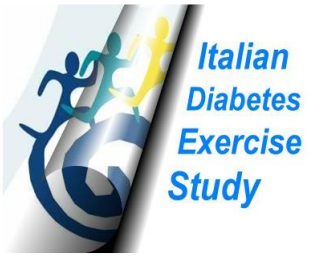
Laboratory of Clinical Chemistry, Sant'Andrea University Hospital, Rome, Italy: Patrizia Cardelli, Stefano Cavallo.

Data Management team

1. Stefano Balducci, MD, Metabolic Fitness Association O.N.L.U.S., Monterotondo, Rome, Italy; coordinator;
2. Elena Alessi, MD, Diabetes Unit, Sant'Andrea University Hospital, Rome, Italy: responsible for control of data completeness, coherence, and plausibility;
3. Gianluca Balducci, Phisioterapist, Metabolic Fitness Association O.N.L.U.S., Monterotondo, Rome, Italy: responsible for monitoring of adverse events during theoretical and practical counseling sessions;
4. Giorgio Orlando, PhD, Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy: responsible for estimation of VO_{2max}, analysis of self-reported MS questionnaire;
5. Silvano Zanuso, PhD, Centre for Human Performance and Sport, University of Greenwich, Chatham Maritime, UK: responsible for calculation of PA and SED-time from accelerometer readings;
6. Patrizia Cardelli, PhD, Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, and Laboratory of Clinical Chemistry, Sant'Andrea University Hospital, Rome, Italy: responsible for biochemical tests;
7. Giuseppe Lucisano, StatD, Clinical Pharmacology and Epidemiology, Consorzio Mario Negri Sud, S. Maria Imbaro, Italy: responsible for analysis of well-being/depression and QoL questionnaires.

Prospective, open-label, parallel, randomized clinical superiority trial comparing a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle versus standard care in sedentary and physically inactive patients with type 2 diabetes

The Italian Diabetes and Exercise Study 2 (IDES_2)



Study protocol

Final version; 15.09.2012*

Submitted to the Ethics Committee of Sant'Andrea University Hospital
for final approval in the session of 25.09.2012

*Ethics Committee approval and trial registration details (including World Health Organization Trial Registration Data Set, see Appendix A) added on 12.10.2012

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Study synopsis

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Study title: The Italian Diabetes and Exercise Study 2: a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle**.**

Acronym: IDES_2.

Registration: ClinicalTrials.gov; NCT01600937; URL https://clinicaltrials.gov/ct2/show/NCT01600937.

Ethical approval: obtained from the Ethics Committee of Sant'Andrea University Hospital on 25.09.2012.

Type of study: Prospective, open-label, parallel, randomized controlled trial.

Condition: Type 2 diabetes.

Objective: To compare a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle versus standard care in sedentary and physically inactive patients with type 2 diabetes.

Intervention: Structured counseling consisting of one individual theoretical session, held by a physician (diabetologist), and eight twice-a-week individual theoretical and practical sessions, held by a certified exercise specialist, once a year for three years, on top of standard care.

Key inclusion and exclusion criteria:

a. Inclusion criteria:
- Known T2DM (defined by the ADA criteria) of at least 1-year duration;
- Age 40-80 years;
- BMI 27-40 kg/m^2;

- Sedentary lifestyle (i.e., more than 8 hours/day spent in any waking behavior 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;
- Ability to walk 1.6 Km without assistance;
- 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);
- Conditions not specifically mentioned above at the discretion of the clinical site.

Participating centers: 3.

Sample size: 300 (284 + 5% expected drop-out).

Enrollment: 18 months (expected).

Trial duration: 36 months.

Endpoints:

a. Primary: Objectively-measured physical activity and sedentary time.

b. Secondary:

- Physical fitness: cardio-respiratory fitness, muscle strength, flexibility;
- Modifiable cardiovascular risk factors and scores: HbA_{1c}, lipid profile, blood pressure, high-sensitivity C-reactive protein; UKPDS total and fatal CHD and stroke 10-year risk scores;
- Musculoskeletal disturbances;
- Well-being/depression;
- Health-related quality of life.

Background

It is predicted that by the year 2030, about 552 million people worldwide will be diagnosed with diabetes mellitus (DM), mainly type 2 DM (T2DM) [1]. This epidemic of T2DM is causally related to the rising incidence of obesity [2], with the increased calorie consumption combined with reduced energy expenditure contributing to the development of both conditions [3]. The diverging trends of decreasing energy intake and increasing body weight suggest that reduced physical activity (PA) may be the major determinant of the dramatic increase of obesity levels observed during the last decades among US adults [4]. In fact, a recent report from the National Health and Nutrition Examination Survey (NHANES) indicates that less than 50% of US adults achieve the recommended level of PA and ~30% of them engage in no PA at all, though projections suggest an improvement [5]. The burden of physical inactivity is even greater among people with T2DM or those at highest risk for developing this condition, who are well below the national norms for PA [6]. Therefore, PA is now considered as a cornerstone of T2DM management, together with diet and medication [7,8].

The joint position statement of the American College of Sports Medicine (ACSM) and the American Diabetes Association (ADA) [9] recommends that individuals with T2DM regularly perform moderate-to-vigorous-intensity PA (MVPA), i.e., at least 150 min/week of aerobic exercise plus at least 2–3 sessions/week of resistance training. In addition, the ACSM/ADA guidelines recommend to train under the supervision of qualified exercise trainers, particularly when undertaking resistance exercise, to ensure optimal health benefits and to minimize injuries, and encourage to increase total daily unstructured (commuting, occupational, home, and leisure-time [LT]) PA. These recommendations are based on a large body of experimental evidence which has been accumulating during the last decade. Two randomized controlled trials (RCTs), the Diabetes Aerobic and Resistance Exercise (DARE) study [10] and the Health benefits of Aerobic and Resistance Training in individuals with type 2 Diabetes (HART-D) study [11], have demonstrated that combined aerobic and resistance training is more effective than either one alone in reducing hemoglobin (Hb) A_{1c} in patients with T2DM. A systematic review and meta-analysis including the DARE and HART-D studies showed that structured exercise training, either aerobic, resistance, or both, is associated with HbA_{1c} reduction in patients with T2DM, especially if of more than 150 min/week and when combined with dietary advice [12]. Furthermore, another RCT, the Italian Diabetes and Exercise Study (IDES), showed that a strategy combining a supervised mixed (aerobic and resistance) exercise training program with structured exercise counseling was more effective than counseling alone in ameliorating HbA_{1c} and other modifiable cardiovascular risk factors, reducing coronary heart disease (CHD) 10-year risk scores, decreasing the number and/or dosage of medications, and improving physical fitness and quality of life (QoL) in a large cohort of sedentary subjects with T2DM [13-15].

The ACSM/ADA guidelines also recommend that T2DM individuals decrease the amount of time spent in daily sedentary behavior and interrupt prolonged sitting with bouts of light-intensity PA (LPA) every 30 min [9]. These recommendations, which are additional to, and not a replacement for, increased structured MVPA/exercise, are based on recent findings indicating that sedentary behavior exerts a detrimental effect on metabolic health through pathways distinct from those of MVPA [16,17]. In fact, higher sedentary (SED)-time in individuals with T2DM was found to be associated with higher metabolic risk, independent of time spent in MVPA [18]. In addition, Healy *et al.* reported that increased breaks in SED-time were beneficially associated with metabolic profile, independent of MVPA and also of total SED-time [19]. However, compliance with PA/exercise recommendations is generally difficult for a number of external and internal barriers [20]. According to the ecological model of active living [21] and sedentary behavior [22], time spent in these behaviors across different domains (i.e., leisure-time, transport, household, and occupation) is determined by both extrapersonal (i.e., family, socio-cultural, policy, built, and natural environment) and intrapersonal (i.e., health status, motivation, self-efficacy) factors. Progressive urbanization is associated with increasing physical inactivity and sedentariness [23], thus requiring multisectoral public policies and initiatives to create urban environments that help people to be more physically active and less sedentary [24]. In addition, individuals suffering from obesity and T2DM are usually well below the recommended level of PA and spend a large amount of daily hours as SED-time [6], thus indicating the need for behavioral intervention strategies targeting both promotion of PA and reduction of SED-time. Unfortunately, to date, effective, acceptable, feasible, and validated strategies to

adopt and particularly maintain a physically active lifestyle are lacking. On the one hand, supervised exercise interventions, though successful in increasing MVPA [13], may not be adequate to reduce SED-time and might even trigger compensatory sedentary behavior [25]. Moreover, they are expensive in terms of time and costs and may not be suitable for implementation in routine clinical practice. On the other hand, available studies with counseling-based interventions are very heterogeneous in terms of theory and techniques of behavior change, modalities of intervention delivery, and domains of behavior targeted [26,27]. Furthermore, these studies generally involved small samples for short time periods and, in most of them, changes in PA were not objectively measured, as they were derived from self-report measures, which are imprecise and do not accurately capture SED-time and particularly LPA [28]. Thus, to date, there is no clear evidence that changes in PA/sedentary behavior can be maintained in the long-term and this hampers the validation and implementation of PA/exercise as a therapy for T2DM. In fact, though observational studies have suggested that increasing PA has beneficial effects on all-cause and cardiovascular mortality [29-31], a cause-effect relationship can be derived only from clinical trials of adequate duration in which adherence to PA/exercise is maintained throughout the study. The Look AHEAD (Action for Health in Diabetes) is an ongoing trial testing the effect of an intensive lifestyle intervention designed to achieve and maintain weight loss on cardiovascular morbidity and mortality in overweight patients with T2DM; this intervention is multicomponent and includes, but is not limited to, an increase in PA, mainly MVPA (with a goal of $\geq$175 min/week) [32]. Unfortunately, data from a subgroup of patients indicate that the marked increase in cardio-respiratory fitness observed at year one was significantly diminished after four years, in parallel with the decreasing intensity of the intervention [33], thus limiting the interpretation of the results with respect to the effect of PA.

In the IDES, supervised training, in addition to providing significant health benefits, was also successful in promoting PA (mainly LPA) outside the supervised sessions [13], likely by improving patient's knowledge, confidence, and sureness in his/her ability to perform PA effectively and safely on his/her own. This finding prompted us to design a behavioral intervention strategy derived from the original IDES protocol and aimed at producing a sustained behavior change including both increased PA and decreased SED-time.

Aim

The IDES_2 is aimed at investigating whether this behavioral intervention strategy is superior to current approach, i.e., standard medical care including general physician recommendations, in adopting and maintaining a physically active lifestyle in patients with T2DM, by increasing total daily PA and reducing SED-time. This strategy combines the seven-step theoretical exercise counseling [34] provided to all IDES participants [35] with eight bi-weekly sessions, supervised by an exercise specialist in a dedicated gym facility. These supervised sessions, which in the IDES were administered to the intervention group for the entire 12-month study duration [35], serve as a theoretical and practical counseling to promote and maintain a physically active behavior. Intervention is repeated every 12 months for three years and PA and SED-time are objectively monitored throughout the study by the use of an accelerometer.

Research design and Methods

Design

The IDES_2 is a prospective, open-label, parallel, randomized controlled superiority trial comparing a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle versus standard care in sedentary and physically inactive patients with T2DM.

Ethics

The study complies with the Declaration of Helsinki. The research protocol (final version, September 15, 2012) has been approved by the Ethics Committee of Sant'Andrea University Hospital (Comitato Etico dell'Azienda Ospedaliera Sant'Andrea) on September 25, 2012 (Prot. n. 212/2012) and has been registered with ClinicalTrials.gov on October 10, 2012 (NCT01600937; URL https://clinicaltrials.gov/ct2/show/NCT01600937) (see **Appendix A**). Written informed consent (see

Appendix B) will be provided by each participant. Participants will not be provided with an honorarium. Participation is free of charge. The flow chart of the study is presented in **Figure 1**.

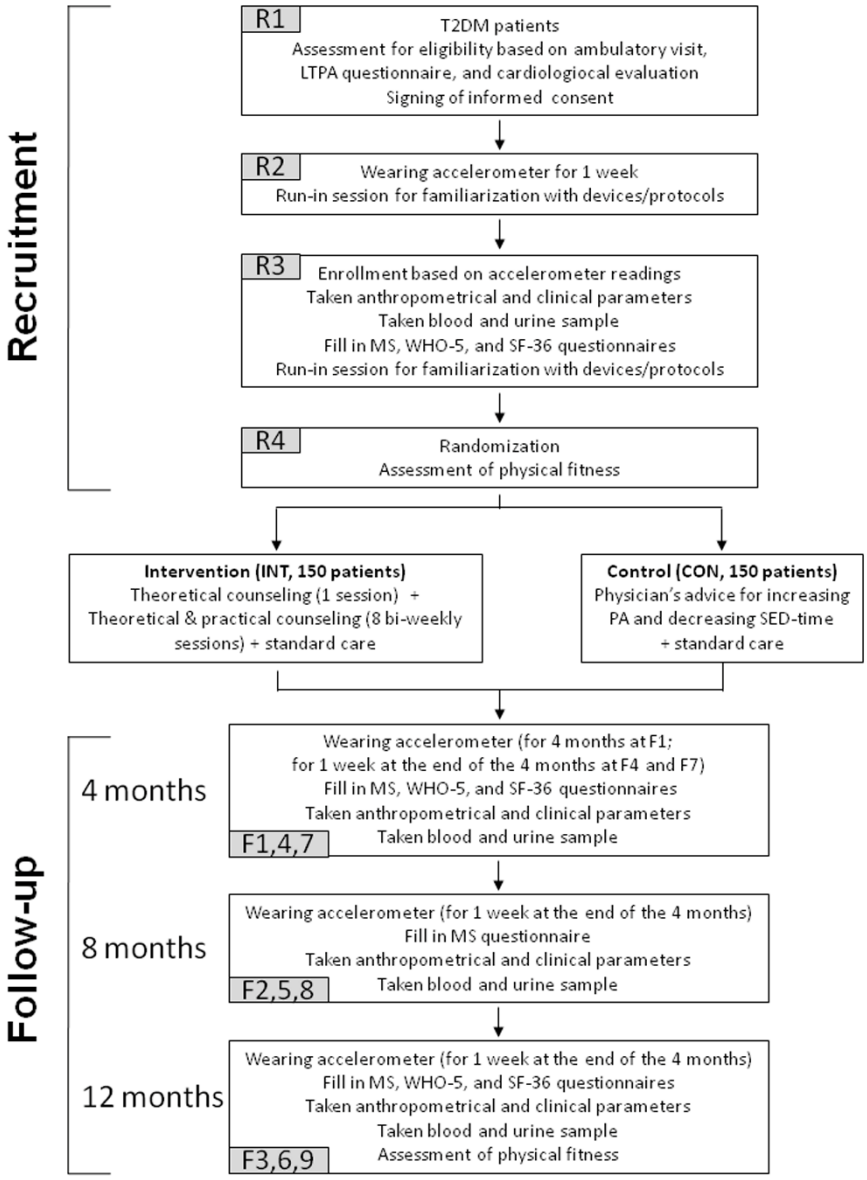

Figure 1. Study flow-chart. Sequence of recruitment and follow-up visits in the IDES_2. T2DM = type 2 diabetes mellitus; LTPA = leisure-time physical activity; MS = musculoskeletal; WHO = World Health Organization; SF = Short Form; INT = intervention group; CON = control group; PA = physical activity; SED-time = sedentary time.

Participants

The main entry criterion is known T2DM (defined by the ADA criteria [36]) of at least 1-year duration. Additional requirements are age 40-80 years, body mass index (BMI) 27-40 kg/m^2, physically inactivity (i.e., insufficient amounts of PA according to current guidelines [37]) and sedentary lifestyle (i.e., more than 8 hours/day spent in any waking behavior characterized by an energy expenditure ≤1.5 metabolic equivalents [METs] while in a sitting or reclining posture [38]) from at least 6 months, ability to walk 1.6 Km without assistance, and eligibility after cardiologic evaluation.

In order to preserve high internal validity and reduce risk of adverse events, the criteria presented 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.

Table 1. Exclusion criteria. Criteria for excluding patients for entry into the IDES-2. BP = blood pressure; eGFR = estimated glomerular filtration rate; HR = heart rate; NYHA = New York Heart Association; ECG = electrocardiogram.

- 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 (systolic) or >10 mmHg (diastolic)
- 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 (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
- Conditions not specifically mentioned above at the discretion of the clinical site

Investigators

A specific strategy has been implemented to train physicians and exercise specialists involved in the IDES and IDES_2 in order to standardize procedures, prevent clustering effect, improve efficacy and safety of intervention, promote patient adherence, and minimize drop-out.

The training path dedicated to physicians (diabetologists) was aimed at increasing their awareness of the importance of PA/exercise as a preventive and therapeutic measure in T2DM and other metabolic disorders as well as at instructing them on how to administer a structured exercise counseling and prescribe PA/exercise to subjects suffering from these disease conditions.

The training path dedicated to exercise specialists was aimed at instructing professionals with a Bachelor degree in exercise science on how to assess physical fitness and PA volume and to supervise exercise sessions, including monitoring of clinical parameters, in individuals with metabolic disorders. At the end of the training program, exercise specialists were certified for assisting these patients in their participation to exercise training programs.

All these activities were proposed and managed by the Metabolic Fitness Association and were detailed in the on-line supplemental material of a previous IDES publication [13].

To minimize dropout and reduce the attrition bias due to missing data, both physicians and exercise specialists were specifically instructed on how to promote participant retention in the trial. In particular, they were recommended to contact participants at regular intervals. Moreover, investigators were instructed 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 treatment. This strategy was successfully applied to the IDES, in which adherence was high and drop-out was low [13].

Recruitment

Three-hundred patients will be recruited in three tertiary referral outpatients Diabetes Clinics in Rome (see **Appendix C**). All patients attending these clinics will be evaluated for eligibility. The recruitment process includes four visits designated as R1, R2, R3, and R4 (**Figure 2 and Table 2**).

On R1, patients fulfilling the above criteria will be identified in each Diabetes Clinic by the Diabetologist based on medical history, clinical examination, and results of the Minnesota LTPA questionnaire. Then, patients will be asked to sign a statement of informed consent (see **Appendix B**), will be registered in the IDES_2 database available at http://www.metabolicfitness.it/, and will undergo a cardiologic examination for cardiovascular evaluation, including a resting ECG and, based on clinical judgment, an echocardiogram and/or an ECG treadmill test.

On R2, in order to objectively verify sedentary habits and physical inactivity, eligible patients will be asked to wear an accelerometer [39,40] for 1 week and to report on a daily diary the hours spent wearing the instrument and those spent sleeping and napping, and performing non-accelerometer recordable PAs. Then, patients will attend a run-in session for familiarization with testing devices and protocols for the assessment of physical fitness in the gym facility connected with the Diabetes Clinic.

On R3, patients will be enrolled by the Diabetologist after confirming eligibility on the basis of the accelerometer readings. Then, baseline anthropometrical and clinical parameters and blood and urine samples for biochemical testing will be taken and, based on the results of these tests, patients will receive a standard treatment regimen including nutritional therapy and prescription of pharmacological agents as needed. Subsequently, participants will be asked to fill in three questionnaires: a 50-item self-report questionnaire for musculoskeletal (MS) symptoms [Balducci *et al*. Unpublished observations], the World Health Organization (WHO)-5 Well-Being Index, a five-item questionnaire for screening of depression [41]; and the Short Form (SF)-36 health survey, with eight scales and two summary measures evaluating physical and mental health-related QoL [42]. Finally, participants will perform another run-in familiarization session for physical fitness assessment in the gym facility.

On R4 (baseline visit), all participants will be informed by the Diabetologist about group assignment, will receive standard care plus PA advices or the counseling intervention, according to the assigned study group. Then, they will undergo baseline assessment of physical fitness in the gym facility, and will be given the accelerometer to be worn for four months, together with the daily diary to report on the hours spent wearing the instrument, sleeping and napping, and performing non-accelerometer recordable PAs.

Randomization

Patients will be randomized 1:1 to an intervention (INT) group (n=150), receiving a theoretical and practical exercise counseling on top of standard care, and a control (CON) group (n=150), receiving only standard care including general physician recommendations for increasing daily PA and reducing SED-time.

Randomization will be stratified by center and, within each center, by age ($<$ or $\geq$ 65 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 Department of Clinical Pharmacology and Epidemiology, Consorzio Mario Negri Sud, S. Maria Imbaro, Italy, and implemented by central telephone.

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. However, to minimize potential bias due to differential administration of standard care, physicians will regulate glucose-, lipid- and blood pressure (BP)-lowering treatment according to an objective target-driven protocol.

Follow-up

Participants from both groups will attend nine follow-up visits, F1 to F9, at month 4, 8, 12, 16, 20, 24, 30, and 36, respectively (**Figure 2 and Table 2**).

On F1, patients will return the key and daily diary, anthropometrical and clinical parameters and blood and urine samples will be taken, and patients will be asked to fill in the MS, WHO-5, and SF-36 questionnaires. Three-to-five days later, patients will be seen again for eventual adjustment of dietary and pharmacological prescriptions, based on clinical and laboratory data.

On F2, F3, F4, F5, F6, F7 and F8, anthropometrical and clinical parameters and blood and urine samples will be taken; patients will be asked to fill in the MS, WHO-5, and SF-36 questionnaires (the latter two only at F3, F4, F6 and F7) and will be given the accelerometer (to be worn for seven days), together with the daily diary. Seven-to-ten days later, patients will be seen again to collect the key and daily diary and for eventual adjustment of dietary and pharmacological prescriptions, based on clinical and laboratory data. On F3 and F6 only, participants will repeat the assessment of physical fitness in the gym facility.

On F9, patients will be given the accelerometer (to be worn for seven days), together with the daily diary. Seven-to-ten days later, patients will be seen again to collect the key and daily diary and to obtain anthropometrical and clinical parameters and blood and urine samples. Finally, they will be asked to fill in the MS, WHO-5, and SF-36 questionnaires and will undergo evaluation of physical fitness in the gym facility.

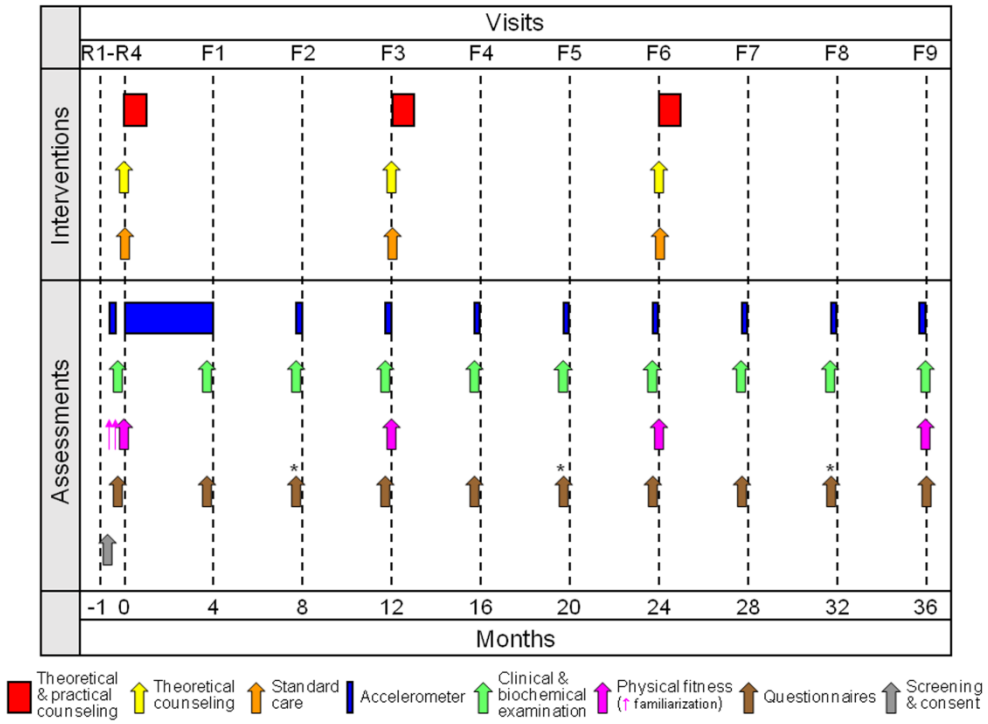

Figure 2. Study timeline. Timeline of the interventions and assessments in the IDES_2. * Only MS questionnaire.

Table 2. Study schedule. Overview of the study visits and assessments in the IDES_2. * Three-to-ten days after the visit. LTPA = leisure time physical activity; CV = cardiovascular; MS = musculoskeletal; WHO = World Health Organization; SF = Short Form; CON = control group; INT = intervention group.

	Study period															
	Recruitment and allocation				**Follow-up**											
	Recruitment			**Allocation**	**Post-allocation**											**Close-out**
Visit	**R1**	**R2**	**R3**	**R4**	**yr 1**	**F1**	**F2**	**F3**	**yr 2**	**F4**	**F5**	**F6**	**yr 3**	**F7**	**F8**	**F9**
Time	**-3 wks**	**-2 wks**	**-1 wks**	**0**	**mo 1**	**mo 4**	**mo 8**	**mo 12**	**mo 13**	**mo 16**	**mo 20**	**mo 24**	**mo 25**	**mo 28**	**mo 32**	**mo 36**
Enrolment																
Eligibility screen																
Medical history	X															
Clinical examination	X															
LTPA questionnaire	X															
Confirmation of sedentary habits/ physical inactivity			X													
Informed consent	X															
Demographics	X															
Cardiologic evaluation	X															
Allocation				X												
Interventions																
CON group																
Physician's advice for increasing daily PA and reducing SED-time				X					X				X			
INT group																
Theoretical session				X				X				X				
Theoretical and practical sessions					X				X				X			

Both: Standard care																
Prescription				X												
Adjustment						X*	X*	X*		X*	X*	X*		X*	X*	
Assessments																
Physical fitness																
Run-in familiarization session		X	X													
Assessment				X				X				X				X
Accelerometer (key)																
Delivery of the key (and daily diary)		X		X			X	X		X	X	X		X	X	X
Return of the key (and daily diary)			X			X	X*	X*		X*	X*	X*		X*	X*	X*
CV risk factors																
Anthropometric data			X			X	X	X		X	X	X		X	X	X
Bio-impedance			X			X	X	X		X	X	X		X	X	X
Blood pressure			X			X	X	X		X	X	X		X	X	X
Blood & urine testing			X			X	X	X		X	X	X		X	X	X
Questionnaires																
MS			X			X	X	X		X	X	X		X	X	X
WHO-5			X			X		X		X		X		X		X
SF-36			X			X		X		X		X		X		X

Post-trial follow-up

The vital status will be verified by interrogating the Italian Health Card database (https://sistemats4.sanita.finanze.it/simossHome/login.jsp) and, in case of death, the cause will be retrieved from medical records obtained from patient's general practitioner and/or relatives.

Intervention

The intervention in the INT group consists of aggregated behavior change techniques (one individual theoretical counseling session plus eight individual theoretical and practical counseling sessions), once a year for three years.

This approach is derived from the original IDES protocol [35], has been designed based on the Social Cognitive Theory and Health Belief Model, and uses several behavior change techniques [43] (**Table 3**).

Table 3. Characteristics of behavioral intervention. Theories of behavior change, behavior change techniques, and mode of delivery of intervention in the IDES_2. Number in squared brackets corresponds with the code assigned to each technique in ref #43.

	Theoretical exercise counseling session	**Theoretical and practical exercise counseling sessions**
Theory of behavior change	Social Cognitive Theory Health Belief Model	Social Cognitive Theory Health Belief Model
Behavior change techniques	Provide information on consequences of behavior in general and individual [1-2] Goal setting [behavior][5] Goal setting [outcome] [6] Barrier identification/problem solving [8] Set graded tasks [9] Prompt review of behavioral goals [10] Prompting generalization of a target behavior [15] Prompt self-monitoring of behavior[16] Provide information on where and when to perform the behavior [20] Motivational interviewing [37]	Provide information on consequences of behavior in general and individual [1-2] Goal setting [behavior][5] Goal setting [outcome] [6] Action planning [7] Barrier identification/problem solving [8] Prompt review of behavioral goals [10] Prompt review of outcome goals [11] Prompting generalization of a target behavior [15] Prompt self-monitoring of behavior[16] Prompt self-monitoring of behavioral outcome [17] Provide information on where and when to perform the behavior [20] Provide instruction on how to perform the behavior [21] Model/Demonstrate the behavior [22] Time management [38]
Delivery of the intervention	Individual face-to-face session Physician interventionist One 30-min session once a year for three years	Individual face-to-face sessions Exercise specialist interventionist Eight 75-min sessions once a year for three years

It has been designed to promote a two-step behavior change, i.e., (1) decreasing SED-time by substituting it with a wide range of LPAs and/or interrupting prolonged sitting at home or work with brief bouts of LPA; and (2) reallocating time spent in sedentary behavior and/or LPA towards gradually increasing amounts of purposeful MVPA. The rationale behind this approach is that substituting LPA for SED-time would increase patient's physical ability, self-efficacy, and motivation, thus allowing him/her to engage safely and effectively in MVPA.

A detailed checklist of the procedures will be made available to care providers in order to assure strict adherence with the protocol.

Theoretical counseling sessions

The theoretical individual, face-to-face counseling session has been previously validated [34] and tested successfully in clinical settings, including the IDES [13,44]. This session lasts 30 minutes, will be held in each Diabetes Clinic, and will be conducted by a trained diabetologist through the seven steps presented in **Table 4** [34]. It is focused on both SED-time/LPA and MVPA across all settings (i.e., leisure-time, transport, household, and occupation). Specifically, it is aimed at (a) assessing patient's current behavior; (b) increasing his/her awareness of the importance of targeting both domains of PA/sedentary behavior; (c) setting individual goals and programs; (d) identifying internal and external barriers to behavior change in the patient's personal, family, social, work, and environmental context; and (e) discussing practical solutions for the problems identified.

Table 4. Steps of the theoretical counseling session. List of items covered by the diabetologist during the session. PA = physical activity; SED-time = sedentary time; T2DM = type 2 diabetes mellitus.

#	Item	Content
1	**Motivation**	The benefits of PA and harms of sedentary behavior are described as reported by the scientific literature, stressing those appealing most to each patient. Efforts are designed to convince the patient that increasing PA and reducing SED-time is the pre-eminent cure for T2DM as well as to understand what positive expectations the individual patients held from this change in behavior.
2	**Self-efficacy**	Self-efficacy is promoted by patient collaboration in designing an individualized program for increasing PA / reducing SED-time, based on age and physical state and setting realistic personal goals.
3	**Pleasure**	Based on the patient's previous experience of PA, a choice of several interchangeable indoor and outdoor PAs in all settings (i.e., leisure-time, transport, household, and occupation) is proposed to identify those that are more appealing.
4	**Support**	The supportive presence of a partner/family member/group of peers is preferred.
5	**Comprehension**	Feedback from the patients is elicited to check if they really understand the valuable advantages of the behavioral change. After the PA program is established, the patient is questioned to establish whether there is a really positive attitude toward the behavioral change. Care is taken to recognize uncertainties and identify perceived impediments to increasing PA and reducing SED-time.
6	**Lack of impediments**	Potential obstacles to regular PA and change in sedentary behavior are identified. Instead of simply suggesting a solution, patients are invited to solve the problem and their proposals are supplemented with advice on time management strategies.
7	**Diary**	The patients are asked to record daily the type and time of PA they perform and the time they spend in sedentary behavior. On the next yearly counseling session, the diary is used to assess the amount of PA/SED-time (together with accelerometer readings), to encourage patients' self-efficacy, to increase the time or frequency of PA, and to overcome practical problems related to increasing PA and reducing SED-time.

Theoretical and practical counseling sessions

The theoretical and practical counseling intervention program consists of eight, distinct but interrelated modules that cover topics such as DM, free living PA and exercise, sedentary behavior, and the psychology of behavior change and are geared to expand and put into practice the knowledge acquired during the theoretical counseling session. The sessions will be held twice-a-week by a certified exercise specialist in three specialized gym facilities, each connected with one of the three Diabetes Clinics (see **Appendix C**).

Each supervised exercise session consists of 30 minutes of aerobic exercise followed by 30 minutes of resistance exercise, both at low-to-moderate-intensity depending on the patient's physical ability, plus

additional 15 minutes for warm up and cool down (including stretching). Moreover, the exercise specialist improves patient's knowledge of the effects of exercise on patient's own health, including both advantages and disadvantages; conditions under which exercise should not be done; difference between habitual and occasional exercise; essential parameters of wellness such as BP, heart rate (HR), and blood glucose. In addition, health instructions are given to identify the difference between aerobic and resistance exercise; evaluate the intensity of exercise; perform warm-up and stretching safely; monitor blood glucose correctly; and correct imbalances during and after the exercise session. Finally, the exercise specialist reinforces the message to be less sedentary by increasing the time spent in LPA and eventually MVPA and examine with the patient when and how he/she could substitute PA for sitting time or break it with bouts of PA in all settings (i.e., leisure-time, transport, household, and occupation) and taking into account the patient's family, socio-cultural, policy, built, and natural environment [25]. A detailed list of the actions performed by the exercise specialist during the theoretical and practical counseling sessions is presented in **Table 5**.

Table 5. Checklist of the theoretical and practical counseling sessions. List of the actions performed by the exercise specialist during the sessions. BP = blood pressure; HR = heart rate; PA = physical activity; SED-time = sedentary time.

#	Action
1	Establishes clinical condition and physical fitness and identifies the adequate exercise protocol (using a home-made exercise algorithm), on the basis of previous evaluations.
2	Measures BP, HR, and glucose level before and after each exercise session and provides feedback to the subject on the effect of the specific exercise adopted.
3	Instructs the patient to perform these measurements, indicates the range of glycemic values allowing or contraindicating exercise, and suggests to start with resistance exercise when glycemia is on the low side of the range, especially for those treated with insulin or secretagogues.
4	Reviews with the patient the structure of the exercise session (warm-up, exercise, and cool-down phases), instructs on how to perform a safe warm-up and cool-down, providing practical examples for the different exercise types, and stressing the importance of the gradualism of the increase and decrease of the intensity for the warm up and cool-down, respectively.
5	Explains the difference between aerobic and resistance exercise, proving practical examples of the different exercise forms (endurance exercise machines, resistance exercise devices, free body exercises).
6	For aerobic exercise training, illustrates the correct exercise progression and control and, throughout the counseling sessions, encourages the patients to progressively increase the exercise intensity, instructing on how to identify and control light, moderate and vigorous intensity on the basis of breathing frequency (talk test), rate of perceived exercise, and HR. As an example, exercise intensity is explained on the basis of the ability to perform a conversation as follows: o light intensity: "allows to respond to conversation without problems"; o moderate intensity: "allows to carry on a conversation but with some difficulties"; o vigorous intensity: " the speech is limited to short phrases".
7	Especially for unsupervised PA/exercise and for patients with complications, advices the patients to work at low-to-moderate intensity, as it is the one allowing to reduce risk of adverse events while providing substantial benefits.
8	With regard to the volume of aerobic exercise, gives examples on how to comply with the exercise recommendations on the minimum amount of PA/exercise providing health benefits, and stressing that adding more PA/exercise will results in additional benefits.
9	Describes the correct way of increasing training volume, augmenting first duration and then intensity, especially in patients with complications.
10	Explains the difference between weight bearing and non-weight bearing exercise and the relevance for the subjects with complications, providing practical examples.

11	For resistance exercise training, provides examples on the exercise taxing the major muscle groups (8-10 exercises), explains (and checking the learning of) the correct exercise technique, and illustrates a typical sequence of resistance exercises, alternating opposing muscle groups and/or upper and lower body exercises.
12	Explains the concept that a higher repetitions number corresponds to a light weight to be lifted (therefore lighter intensity) and vice versa, and teaches the correct breathing pattern during the different resistance exercises adopted, reminding that breath holding (Valsalva maneuver) should be avoided.
13	Instructs to perform multiples sets (e.g., 2-3 sets) with adequate recovery between them (e.g., 2 min), depending on the intensity adopted (less repetitions per set usually equal longer recovery time).
14	Identifies indicated and contraindicated resistance exercises on the basis of the specific patient's complications.
15	Provides examples on how to substitute the typical exercises executed in the gym setting with other forms of PA/exercise to be performed outside the gym, reminding patients that new forms of unsupervised PA/exercise should be adopted after consulting the exercise specialist.
16	Helps the patient to organize a typical working day and weekend in order to find time and space for performing any type of PA in all settings (home, commuting, occupational, and leisure-time), reduce the SED-time, and remove potential obstacles.
17	Encourage the patient to interrupt prolonged sitting with brief bouts of PA (aerobic, resistance, or callisthenic), ideally every 30-60 min.
18	Assists the patient in setting behavior and outcome goals as well as in the choice of the indoor and outdoor PAs that are most appealing and feasible.

In the IDES_2, these supervised sessions will serve as counseling intervention aimed at promoting and maintaining a physically active lifestyle, whereas in the IDES they served as exercise intervention, as participants were engaged in the training program for the entire 12-month study duration (and exercised at increasing intensity) [35].

The rationale for using these sessions as theoretical and practical counseling is that, in the IDES, in addition to providing significant health benefits, this intervention was successful also in promoting PA (mainly LPA) outside the sessions, by improving patient's knowledge, skills, and ability and enhancing the intrapersonal determinants of PA behavior (i.e., health status, self-efficacy, and motivation) [13].

Strengths and limitations of the counseling intervention

The strengths of this behavioral intervention strategy include the solid theoretical grounds [26], the use of several behavior change techniques, including those shown to be associated with intervention efficacy [27], and the focus on both PA and SED-time [16], across all settings (leisure-time, transport, household, and occupation) [21,22].

Potential limitations include the possibility that determinants of behavior change maintenance differ from those of behavior change adoption [45], thus requiring different behavioral strategies. However, further research is required to identify these factors, thus allowing to design more effective interventions for sustained change in PA/sedentary behavior. In addition, though both physicians and exercise specialists assist the patient in the identification of external barriers to behavior change and discuss with him/her about possible solutions, a behavioral intervention strategy cannot remove obstacles due to physical environments and needs to be accompanied by adequate health policy programs [45].

Standard care

Participants from the CON group will receive general physician recommendations for increasing the amount of daily PA and decreasing the SED-time.

All patients will receive a treatment regimen aimed at achieving optimal glycemic, lipid, BP, and body weight targets, as established by current guidelines [36]. This program will eventually include glucose-, lipid- and BP-lowering agents as needed and, when indicated, anti-platelet drugs, plus a dietary prescription.

The dietary prescription will be preceded by a preliminary nutritional evaluation with assessment of BMI and the individual patient requirements and preferences. The diet will contain 55% calories from complex carbohydrates, 30% from fat, and 15% from protein. Since all patients are overweight or obese, they will receive a low-calorie diet (negative balance of 500 kcal/day, but not less than 1,500 kcal in males and not less than 1,200 kcal in females), calculated by adding the estimated energy expenditure from routine and voluntary PA to the basal metabolism estimated using the gas exchange analyzer FitMate (Cosmed, Rome, Italy).

At each intermediate visit (i.e., every four months), diet and pharmacological treatment will be eventually adjusted by the Diabetologist using an objective target-driven protocol, based on reported adherence to diet and cardio-metabolic risk profile.

Outcome measures

The primary objective of IDES-2 is to assess the effect of a behavioral intervention versus standard care on the adoption and maintenance of a physically active lifestyle in sedentary and physically inactive patients with T2DM.

Secondary objectives include the assessment of the effect of intervention on physical fitness, modifiable cardiovascular risk factors, MS disturbances, well-being/depression, and health related QoL.

Ancillary objectives include the assessment of the percentage of participants achieving pre-specified targets for PA volume (>20 METs-hour·week^{-1}), LPA (>6 hours·day^{-1}), MVPA ($\geq$150 min·week^{-1}), and SED-time (<10 hours·day^{-1}) and the relationship between change in behavior and change in physical fitness, modifiable cardiovascular risk factors, MS disturbances, well-being/depression, and health related QoL.

The effect of the intervention on the primary and secondary endpoints will be analyzed both short-term, after the initial four months, during which the participants will wear the accelerometer continuously, and long-term, at the end of the three-year period.

While physicians and exercise specialists will be aware of group assignment, the assessor of the primary outcome (i.e., PA and SED-time from accelerometer readings; Dr. Silvano Zanuso) will be blinded to group assignment and blinding of blood and urine samples at central laboratory will be achieved using bar codes.

Measurements

Assessment of PA

At screening, the volume of PA will be assessed retrospectively using the Minnesota LTPA questionnaire [46]. At baseline, follow-up and end-of-study, each participant will be outfitted with a uni-axial piezoelectric accelerometer MyWellness Key (Technogym, Gambettola, IT) [39]. The advantages of using the MWK accelerometer relies on the fact that it offers the possibilities of storing 30 days of continuous movement detection and provides accurate measures of the minutes spent at light, moderate, and vigorous intensities and the total volume of PA [40] also in individuals with T2DM [Zanuso *et al*. Unpublished observations]. The device is attached at the waistband in midline of the right anterior hip and detects the minutes spent at light, moderate and vigorous intensities and the total volume of PA [40]. Each participant will wear the device for four months at baseline and for seven consecutive days every four months thereafter. Upon waking (immediately after bathing or showering), participants will be asked to attach the device at the waistband in midline of the right anterior hip and to report on a daily diary the hours spent wearing the instrument, sleeping and napping, and performing non-accelerometer recordable PAs such as swimming, cycling, skiing, etc.

Participants will be asked to wear the device all day (except if swimming) up to bedtime, in order to avoid the influence of the "time accelerometer worn", which may vary from patient to patient. In this way, it will be possible to assume that the time patients are awake without wearing the accelerometer is spent in sedentary activities (e.g. take a shower, get dressed), unless spent in PAs that cannot be performed while wearing the accelerometer (e.g. swimming). Total SED-time, i.e., the daily time the patients are awake without being engaged in a PA, will be then calculated by adding this time to that recorded by the accelerometer with readings <100 counts/min, a threshold which corresponds with sitting, reclining, or lying down, i.e., to <1.5 METs [47].

Matthews' cut-points will be used to identify time spent in light intensity (100–1951 counts/min corresponding to 1.5–2.9 METs) [48], whereas Freedson's cut-points will be used to determine time spent

in PA of moderate intensity (1952–5724 counts/min corresponding to 3–5.9 METs) and of vigorous intensity (≥5725 counts/min corresponding to ≥6 METs) [49]. Time spent in non-accelerometer recordable PAs, as reported on the daily diary, will be added to that recorded by the accelerometer, according to the intensity of each activity [50]. Moderate-intensity PA will be combined with vigorous-intensity PA into MVPAs, as participants spend little time in vigorous-intensity PA.

Volume of PA will be calculated by summing the duration in minutes of PA at each of the three intensities (either recorded by the accelerometer or, for non-accelerometer recordable PAs, derived from the daily diary) multiplied by their average METs intensity. Results will be expressed as METs-hour·week^{-1}.

Assessment of physical fitness

Parameters of physical fitness, i.e., cardio-respiratory fitness, strength and flexibility, will be evaluated at baseline and month 12, 24, and 36 (end-of-study). The tests will be preceded by two consecutive run-in sessions to become familiar with testing devices and protocols.

Cardio-respiratory fitness will be assessed by a maximal treadmill exercise test using a Balke protocol [51]. The treadmill speed will be set at 88 m/min and the grade is 0% during the first minute, 2% during the second minute, then it will be increased every minute by 1%; after 25 minutes, treadmill grade will be kept constant, whereas speed will be increased by 5.4 m/min every minute. Patients will be instructed not to hold the treadmill rails and will be verbally encouraged to reach volitional exhaustion, as reported by the patient or by the physician for medical reasons. The time to exhaustion will be used to estimate maximal oxygen uptake (VO_{2max}), given the high correlation between these two parameters [52].

For strength assessment, isometric muscle strength will be measured by means of a strain gauge tensiometer (Digimax, Mechatronic GmbH, Germany), as previously described [53]. Isometric tests have been chosen since they are reproducible [54], do not require complex execution technique, and can therefore be easily performed by both trained and untrained subjects. Upper body muscle strength will be assessed by maximal voluntary contractions (MVCs) performed at a shoulder press (Technogym) along the sagittal plane, with a 90° and 45° angle at the elbow and between the upper arm and the trunk, respectively. Lower limb muscle strength will be assessed by MVCs performed at a costumed leg extension machine (Technogym). The angle at the knee and the hip will be fixed at 90°. These exercise modalities have been chosen in order to test the muscles chains involved in fundamental activities of daily living. In both tests, subjects will be asked to exert their maximal force with both limbs simultaneously and to maintain it for at least 2-3 seconds before relaxing. For each exercise, three MVCs will be performed, with 3-min rest interval between contractions. Participants will be asked to perform additional contractions if the MVC of their last attempt exceeds the previous by at least 5 %. During the MVCs, verbal encouragement will be provided always by the same investigator for all subjects.

For hip and trunk flexibility assessment, a standard bending test will be executed [35]. Standing on a step with the legs fully extended, patients will be asked to bend the torso forward to try to touch the ground with their fingertips. The test will be performed three times and the distance between the finger and the ground will be measured by the exercise specialist at the third attempt.

Assessment of modifiable cardiovascular risk factors

Modifiable cardiovascular risk factors will be evaluated at baseline, end-of-study, and every four months in-between.

Body weight and height will be measured using scale and stadiometer and BMI will be then calculated as weight (kg)/height (m^2), while waist circumference will be taken at the umbilicus. Body composition will be evaluated by assessing fat mass and fat-free mass by the use of a bioimpedentiometer (Tanita BF664, Vernon Hills, IL, USA). BP will be recorded with a sphygmomanometer after a five-minute rest with the patient seated with the arm at the heart level.

The following biochemical parameters will be measured: HbA_{1c}, fasting plasma glucose (FPG), serum insulin, C-peptide, triglycerides, total, LDL and HDL cholesterol, high sensitivity-C-reactive protein (hs-CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transpeptidase (γ-GT), creatine kinase (CK), complete blood count, uric acid, blood urea nitrogen (BUN), serum creatinine, urinalysis and albumin/creatinine ratio on first-voided urine samples. Biochemical tests will be centralized

at the Laboratory of Clinical Chemistry of Sant'Andrea University Hospital, an accredited and ISO9000 certified structure, using standard analytical techniques (**Table 6**).

Then, the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index will be calculated from FPG and insulin levels [55], whereas glomerular filtration rate (GFR) will be estimated from serum creatinine by the use of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [56]. Global and fatal CHD and stroke 10-year risk scores will be calculated using the United Kingdom Prospective Diabetes Study (UKPDS) risk engine [57].

Table 6. Laboratory tests. Methods for biochemical measurements in the IDES-2. IDES = Italian Diabetes and Exercise Study; HbA_{1c} = hemoglobin A_{1c}; HPLC = high-performance liquid chromatography; FPG = fasting plasma glucose; hs-CRP= high sensitivity-C-reactive protein; AST = aspartate aminotransferase; ALT = alanine aminotransferase; γ-GT = γ-glutamyl-transpeptidase; CK = creatine kinase; BUN = blood urea nitrogen.

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
Insulin	Chemiluminiscent immunometric assays (Immulite 2000 Thes)	Diagnostic Products Corporation, Los Angeles, CA, USA
C-peptide	Chemiluminiscent immunometric assays (Immulite 2000 Thes)	Diagnostic Products Corporation, Los Angeles, CA, 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
LDL 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
AST	VITROS 5,1 FS Chemistry System	Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA
ALT	VITROS 5,1 FS Chemistry System	Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA
γ-GT	VITROS 5,1 FS Chemistry System	Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA
CK	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
BUN	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

Assessment of MS disturbances

Evaluation of MS symptoms will be accomplished by the use of a 50-item self-report questionnaire (see **Appendix D1**) [Balducci *et al*. Unpublished observations], which investigates shoulder, arm, elbow, wrist, hand, spine, hip, knee, ankle, and foot problems.

Assessment of well-being/depression

Screening for depression will be performed by asking participants to fill in the WHO-5 Well-Being Index, a five-item questionnaire (see **Appendix D2**) [41], which was found to be suitable for use in outpatients with type 1 DM and T2DM [58].

Assessment of health-related QoL

Health-related QoL will be evaluated using the Italian version (ISF-36) [59] of the SF-36 health survey (see **Appendix D3**) [42], which yields four physical and four mental health component scores and has been previously validated in subjects with T2DM [60,61]. The physical and mental component summary measures (PCS and MCS, respectively) will be normalized to a population mean of 50 and SD of 10.

Adverse events

Low-to-moderate risks are associated with PA/exercise performed during the theoretical and practical counseling sessions and assessment of physical fitness.

Risk of MS injuries will be minimized as much as possibly through supervision by a certified exercise specialist. Risk of other adverse events including cardiovascular events will be minimized by thorough medical and cardiologic examination before enrollment.

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

Sample size

Sample size has been calculated based on preliminary accelerometer data showing that daily PA in sedentary, physically inactive T2DM patients is 10.5±4.1 METs-hour·week^{-1}.

To observe a 15% increase in daily PA with a statistical power of 90% (α=0.05) by unpaired t-test, 142 patients per arm are needed (284 total). A sample size of 300 patients allows supporting a 5% dropout rate, as observed in the IDES [13].

Statistical analysis

The χ^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 intent-to-treat analysis for primary and secondary endpoints will be applied to all randomized patients with a baseline and at least one post-baseline value. The superiority of the intervention versus standard care on the primary and secondary endpoints will be assessed by a repeated measures analysis of covariance (ANCOVA - using PROC MIXED in SAS software) of change from baseline to the end-of-study. In case of differences between groups in baseline values, we will consider a baseline adjustment to improve power.

Subgroup analyses will be conducted by stratifying participants by gender, age (< versus $\geq$ 65 years) and type of diabetes treatment (non-insulin versus insulin) and eventually according to baseline, end-of-study, or baseline-to end-of study strata of any of the measured variables.

To account for differences in change of medication throughout the study period between the two groups, which might affect performance with respect to cardiovascular risk factors, both multiple regression and sensitivity analyses we will performed. 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, use adjustment methods for missing data, and conduct a sensitivity analysis to assess the robustness of inferences about treatment effects to various missing-data assumptions [62]. In particular, repeated measures models with an autoregressive correlation type matrix will be applied in order to account for both missingness at random and potential correlation within subjects. These methods are more efficient in that they allow evaluating all subjects, even those with incomplete data [63,64].

To guarantee replicability and avoid outcome selective reporting, a fully specified statistical analysis plan will be written before analysis.

Statistical analyses will be performed by at the Department of Clinical Pharmacology and Epidemiology, Consorzio Mario Negri Sud, using SAS software release 9.3 (Cary, NC, USA).

Risk of bias

As reported above, several methods will be used to minimize risk of any type of bias (**Table 7**).

Table 7. Methods against bias. List of actions taken to minimize risk of bias. BP = blood pressure; PA = physical activity; SED-time = sedentary time.

Type of bias	Action(s)
Selection bias	Random sequence generation Allocation concealment
Performance bias	Objective target-driven regulation of glucose-, lipid- and BP-lowering treatment by physicians
Detection bias	Blinding of assessors of primary outcome (i.e., PA and SED-time from accelerometer readings) and blood and urine testing.
Attrition bias	Keep up to date contact information for contacting participants at regular intervals Collect complete data for both the primary and secondary outcomes, regardless of whether subjects continue to receive treatment
Reporting bias	Preparation of a fully specified statistical analysis plan before analysis

Dissemination policy

Trial results will be communicated to participants via the website of the Metabolic Fitness Association (http://www.metabolicfitness.it/) and by organizing a dedicated event. Results will be also communicated to the scientific community via publication and to the public via press release.

The final report(s) will follow the main CONSORT 2010 guideline as well as its extension to non-pharmacological interventions. The following criteria will be adopted for authorship, according to the ICMJE Recommendations: (a) substantial contributions to the conception and design; or the acquisition, analysis, or interpretation of the data; (b) the drafting of the article or critical revision for important intellectual content; (c) final approval of the version to be published; and (d) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. A professional writer will help with manuscript preparation.

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

Data collection, storage and security

Data from physical fitness measurements, clinical and biochemical assessments (including medications), and questionnaires will be entered separately by two investigators in each Diabetes Clinic into the IDES_2 database and then checked for coherence, completeness, and plausibility by an independent investigator at the Coordinating Center blinded to group assignment (Dr. Elena Alessi). Data from the accelerometers will be downloaded into the IDES_2 database and calculation of PA and SED-time will be centralized at the University of Greenwich (Dr. Silvano Zanuso, who will be blinded to group assignment).

All data will be saved to a password-protected server in the Metabolic Fitness Association and 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.

Participants will be contacted only by personnel directly involved with their care. All computer datasets that contain personal identifiers (e.g. name, address, phone numbers, tax code) will be stored in a separate encrypted dataset using the software encryption option. The pass phrase to gain access to the encrypted files will be known only to the Principal Investigator and the Co-Investigator. Patients' questionnaires will be made anonymous and stored in locked filing cabinets in the Metabolic Fitness Association, together with patients' consent forms.

The Principal Investigator and the Co-Investigator will have access to the final trial dataset and make it available in an anonymized form to the Department of Clinical Pharmacology and Epidemiology, Consorzio Mario Negri Sud, for statistical analysis and eventually, upon request, to other investigators.

Data monitoring and management

The data monitoring and management team, which is responsible for data collection and analysis, is composed by the following members of the IDES_2 Study Group:

1. Stefano Balducci, MD, Metabolic Fitness Association O.N.L.U.S., Monterotondo, Rome, Italy; coordinator;
2. Elena Alessi, MD, Diabetes Unit, Sant'Andrea University Hospital, Rome, Italy: responsible for control of data completeness, coherence, and plausibility;
3. Gianluca Balducci, Phisioterapist, Metabolic Fitness Association O.N.L.U.S., Monterotondo, Rome, Italy: responsible for monitoring of adverse events during theoretical and practical counseling sessions;
4. Giorgio Orlando, PhD, Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy: responsible for estimation of VO_{2max}, analysis of self-reported MS questionnaire;
5. Silvano Zanuso, PhD, Centre for Human Performance and Sport, University of Greenwich, Chatham Maritime, UK: responsible for calculation of PA and SED-time from accelerometer readings;
6. Patrizia Cardelli, PhD, Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, and Laboratory of Clinical Chemistry, Sant'Andrea University Hospital, Rome, Italy: responsible for biochemical tests;
7. Giuseppe Lucisano, StatD, Department of Clinical Pharmacology and Epidemiology, Consorzio Mario Negri Sud, S. Maria Imbaro, Italy: responsible for analysis of well-being/depression and QoL questionnaires.

No *interim* analysis will be conducted.

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.

Support

This work is supported by the Metabolic Fitness Association O.N.L.U.S., Via Nomentana, 27 - 00015 Monterotondo, Rome, Italy; Phone +390690080260; Fax: +390690080235; e-mail: info@metabolicfitness.it. The Metabolic Fitness Association is a Registered Non-Profit Organization that receives voluntary contributions from patients and supports research in the field of physical activity/exercise as a therapy for subjects with metabolic disorders.

The funding body will provide financial support for paying the exercise specialists, the study-specific insurance, the instruments (accelerometer MyWellness Key, gas exchange analyzer FitMate, strain gauge tensiometer Digimax), and the biochemical tests.

The sponsor will have no role 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.

Insurance

For the risk of injuries and other adverse events during the theoretical and practical counseling sessions, patients will be insured through the typical insurance of each gym facility.

Any potential additional risks for the patients caused by study-specific procedures (e.g., theoretical and practical counseling sessions, assessment of physical fitness) will be covered by an additional insurance by the Metabolic Fitness Association O.N.L.U.S. (Insurance-Police-Number: 390-01583709-14010, HDI-Gerling Industrie Versicherung AG, Eisenbahnstr., 1-3, 04315 Leipzig, Deutschland).

Declaration of interests

The Principal Investigator, the Co-Investigator and the components of the Steering Committee declare no financial and other competing interests related to this work.

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Appendix A: World Health Organization Trial Registration Data Set

1. **Primary Registry and Trial Identifying Number:** ClinicalTrials.gov; NCT01600937; URL https://clinicaltrials.gov/ct2/show/NCT01600937.
2. **Date of Registration in Primary Registry:** October 10, 2012
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**, MD, PhD, Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, and Diabetes Unit, Sant'Andrea University Hospital, Via di Grottarossa, 1035-1039, 00189 Rome, Italy; Phone: +39-0633775440; Fax: +39-0633776327; E-mail: giuseppe.pugliese@uniroma1.it.
9. **Public Title:** The Italian Diabetes and Exercise Study 2 (IDES-2): a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle.
10. **Scientific Title:** The Italian Diabetes and Exercise Study 2 (IDES-2): a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle.
11. **Countries of Recruitment:** Italy.
12. **Health Condition(s) or Problem(s) Studied:** type 2 diabetes mellitus.
13. **Intervention:**
    a. Intervention arm
        - Name: Aggregated behavior change techniques.
        - Description: one individual theoretical counseling session, held by a physician (diabetologist) plus eight twice-a-week individual theoretical and practical counseling sessions, held by a certified exercise specialist, once a year for three years, in addition to standard care.

    b. Control arm: general physician recommendations for increasing the amount of daily PA and decreasing sedentary (SED)-time in addition to standard care.
14. **Key Inclusion and Exclusion Criteria**
    a. Inclusion criteria: known T2DM (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 behavior 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, 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); ECG treadmill test suggestive of myocardial ischemia; 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
    - Assignment: parallel
    - Purpose: testing the efficacy of a behavior change strategy in increasing total daily PA and reducing SED-time in patients with T2DM

    c. Phase: NA

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

16. **Date of First Enrollment:** October 20, 2012; **date last enrollment:** February 7, 2014.

17. **Target Sample Size:** 300

18. **Recruitment Status:** completed**.**

19. **Primary Outcome(s)**

    - Name: physical activity and sedentary time;
    - Method of measurement: accelerometer and daily diary;
    - Time points: at baseline and every 4 months for 36 months.

20. **Key Secondary Outcomes**

1. Name: physical fitness; methods of measurement: maximal treadmill exercise test, isometric muscle strength test; bending test; time points: at baseline and every 12 months for 36 months.
2. Name: modifiable cardiovascular risk factors; methods of measurement: clinical and biochemical testing; time points: at baseline and every 4 months for 36 months.
3. Name: musculoskeletal disturbances; method of measurement: self-report questionnaire; time points: at baseline and at 4, 12, 16, 24, 28 and 36 months.
4. Name: well-being/depression; method of measurement: WHO-5 Well-Being Index; time points: at baseline and at 4, 12, 16, 24, 28 and 36 months.
5. Name: health-related quality of life; method of measurement: SF-36 health survey; time points: at baseline and at 4, 12, 16, 24, 28 and 36 months.

Appendix B: Patient Consent Form

Patient Consent Form
(art. 13 D.Igs. 196/2003)

TITLE OF THE STUDY: The Italian Diabetes and Exercise Study 2 (IDES-2): a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle

IDENTIFICATION OF THE STUDY

Dear Patient,

The study in which your Diabetologist takes part has as its principal aim to demonstrate that counseling is able to increase compliance to physical activity (PA) and reduce sedentary behavior in the long term (3 years). The research includes the involvement of 300 patients with type 2 diabetes, it will be carried out in Rome, supported by the Metabolic Fitness Association, a non-profit organization.

The intervention consists of a behavioral intervention strategy comprising one individual theoretical counseling session, held by a diabetologist in the Diabetes Clinic, plus eight, twice a week individual theoretical and practical counseling sessions, held by a certified exercise specialist in the gym facility connected with the Diabetes Clinic. The intervention will be repeated three times for three years.

At baseline and every four months, the participant will be asked to:

- wear an accelerometer, i.e., a device which monitor the duration and intensity of PA in order to calculate the time spent in PAs of light, moderate, and vigorous intensity and the sedentary time;
- undergo measurement of routine anthropometrical, clinical and biochemical parameters;
- fill in the questionnaires on musculoskeletal disturbances, well-being/depression, and health-related quality of life.

PROTECTION OF PERSONAL DATA:

Any information that you provide, the collection and processing of which is indispensable to achieving the objectives of this study, will be treated with methods that guarantee absolute privacy, confidentiality and security in compliance with the standards for the protection of personal data and the right to privacy (Law 31 December 1996 n. 675 and subsequent amendments / additions).

You will be identified by a code and any clinical information concerning you will not be disclosed without prior written permission. The data collected will be made up of initials, date of birth, sex and otherwise sensitive clinical data such as disclosing your state of health.

As a person interested in the processing of your personal data, you will have full access to the information held by your doctor with the authority to exercise the rights of cancellation, transformation, integration, updating, rectification and block within the limits provided for by Article 13 of Law 675/96 regarding the protection of personal data. You will not be billed for exams, whose results will be communicated to your family doctor.

Your participation in this study is completely free and your doctor reserves the right to decide not to enroll you as he/she sees appropriate.

BENEFITS OF THE STUDY:

By agreeing to participate in this study, your doctor will be aware of the presence of cardiovascular risk factors to be monitored. Also the knowledge that will be acquired through your participation will be useful both for you and other patients.

If you have any questions or clarifications, please do not hesitate to talk with your doctor. Please sign and date the following page to confirm that you have read all the information provided and have fully understood the purpose of this study and you freely give consent for the treatment of your personal data. The original of this Personal Consent Form will be deposited in the Archives of the Metabolic Fitness Association. You may also request a copy of your Personal Consent Form.

Consent to the treatment of personal data

TITLE OF THE STUDY: The Italian Diabetes and Exercise Study 2 (IDES-2): a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle

I declare to accept the proposal to participate in the study described in this document. I understood all the information and explanations that were given to me and I had enough time to consider whether to make available, for the purposes of this study, clinical data concerning my person. I consent, in accordance with law 675/96 regarding the protection of personal data, so that clinical data concerning me and the study in question are made available and published in the scientific literature. I agree in particular that the processing of my personal data, including those relating to health, is made for the purposes of research in the limits and in the manner described in this Information and Consent Form.

Signature of the patient _____ **date**_____

Surname and name of the patient _____

Medical investigator's signature _____ **date**_____

Medical Investigator' name _____

Appendix C: The IDES_2 Investigators

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11. Giorgio Orlando, PhD, Department of Human Movement and Sport Sciences, ''Foro Italico'' University, Rome, Italy: responsible for estimation of VO_{2max}, analysis of self-reported MS questionnaire;
12. Silvano Zanuso, PhD, Centre for Human Performance and Sport, University of Greenwich, Chatham Maritime, UK: responsible for calculation of PA and SED-time from accelerometer readings;
13. Patrizia Cardelli, PhD, Department of Clinical and Molecular Medicine, "La Sapienza" University of Rome, and Laboratory of Clinical Chemistry, Sant'Andrea University Hospital, Rome, Italy: responsible for biochemical tests;
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Appendix D: Questionnaires

1. Self-reported questionnaire for musculoskeletal (MS) symptoms
2. World Health Organization (WHO)-5 Well-Being Index (1998 version)
3. Short Form (SF)-36 health survey

1. 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 orthopedic 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?

2. WHO-5 Well-Being Index (1998 version)

Please indicate for each of the five statements which is closest to how you have been feeling over the last two weeks.
Notice that higher numbers mean better well-being (Example: If you have felt cheerful and in good spirits more than half of the time during the last two weeks, put a tick in the box with the number 3 in the upper right corner).

#	Over the last two weeks	All of the time	Most of the time	More than half of the time	Less than half of the time	Some of the time	At no time
1	**I have felt cheerful and in good spirits**	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1	☐ 0
2	**I have felt calm and relaxed**	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1	☐ 0
3	**I have felt active and vigorous**	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1	☐ 0
4	**I woke up feeling fresh and rested**	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1	☐ 0
5	**My daily life has been filled with things that interest me**	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1	☐ 0

Scoring:
The raw score is calculated by totaling the figures of the five answers. The raw score ranges from 0 to 25, 0 representing worst possible and 25 representing best possible quality of life.
To obtain a percentage score ranging from 0 to 100, the raw score is multiplied by 4. A percentage score of 0 represents worst possible, whereas a score of 100 represents best possible quality of life.
Interpretation:
It is recommended to administer the Major Depression (ICD-10) Inventory if the raw score is below 13 or if the patient has answered 0 to 1 to any of the five items. A score below 13 indicates poor well-being and is an indication for testing for depression under ICD-10.
Monitoring change:
In order to monitor possible changes in well-being, the percentage score is used. A 10% difference indicates a significant change.

3. SF-36 health survey

SF-36 QUESTIONNAIRE

Name: _____ Ref. Dr: _____ Date: _____

ID#: _____ Age: _____ Gender: M / F

Please answer the 36 questions of the **Health Survey** completely, honestly, and without interruptions.

1. GENERAL HEALTH:

In general, would you say your health is:

☐ Excellent ☐ Very good ☐ Good ☐ Fair ☐ Poor

Compared to one year ago, how would you rate your health in general now?

☐ Much better now than one year ago
☐ Somewhat better now than one year ago
☐ About the same
☐ Somewhat worse now than one year ago
☐ Much worse than one year ago

2. LIMITATIONS OF ACTIVITIES:

The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

Lifting or carrying groceries

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

Climbing several flights of stairs

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

Climbing one flight of stairs

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

Bending, kneeling, or stooping

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

Walking more than a mile

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

Walking several blocks

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

Walking one block

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

Bathing or dressing yourself

☐ Yes, limited a lot ☐ Yes, limited a little ☐ No, not limited at all

3. PHYSICAL HEALTH PROBLEMS:

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

Cut down the amount of time you spent on work or other activities

☐ Yes ☐ No

Accomplished less than you would like

☐ Yes ☐ No

Were limited in the kind of work or other activities

☐ Yes ☐ No

Had difficulty performing the work or other activities (for example, it took extra effort)

☐ Yes ☐ No

4. EMOTIONAL HEALTH PROBLEMS:

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

Cut down the amount of time you spent on work or other activities

☐ Yes ☐ No

Accomplished less than you would like

☐ Yes ☐ No

Didn't do work or other activities as carefully as usual

☐ Yes ☐ No

5. SOCIAL ACTIVITIES:

Emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

☐ Not at all ☐ Slightly ☐ Moderately ☐ Severe ☐ Very Severe

6. PAIN:

How much bodily pain have you had during the past 4 weeks?

☐ None ☐ Very Mild ☐ Mild ☐ Moderate ☐ Severe ☐ Very Severe

During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

☐ Not at all ☐ A little bit ☐ Moderately ☐ Quite a bit ☐ Extremely

7. ENERGY AND EMOTIONS:

These questions are about how you feel and how things have been with you during the last 4 weeks. For each question, please give the answer that comes closest to the way you have been feeling.

Did you feel full of pep?

☐ All of the time
☐ Most of the time
☐ A good bit of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

Have you been a very nervous person?

☐ All of the time
☐ Most of the time
☐ A good bit of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

Have you felt so down in the dumps that nothing could cheer you up?

☐ All of the time
☐ Most of the time
☐ A good bit of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

Have you felt calm and peaceful?

☐ All of the time
☐ Most of the time
☐ A good bit of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

Did you have a lot of energy?
☐ All of the time
☐ Most of the time
☐ A good bit of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

Have you felt downhearted and blue?
☐ All of the time
☐ Most of the time
☐ A good bit of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

Did you feel worn out?
☐ All of the time
☐ Most of the time
☐ A good bit of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

Have you been a happy person?
☐ All of the time
☐ Most of the time
☐ A good bit of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

Did you feel tired?
☐ All of the time
☐ Most of the time
☐ A good bit of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

8. SOCIAL ACTIVITIES:

During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?
☐ All of the time
☐ Most of the time
☐ Some of the time
☐ A little bit of the time
☐ None of the time

9. GENERAL HEALTH:

How true or false is each of the following statements for you?

I seem to get sick a little easier than other people
☐ Definitely true ☐ Mostly true ☐ Don't know ☐ Mostly false ☐ Definitely false

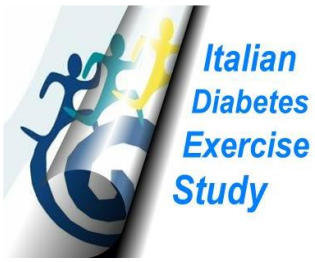
I am as healthy as anybody I know
☐ Definitely true ☐ Mostly true ☐ Don't know ☐ Mostly false ☐ Definitely false

I expect my health to get worse
☐ Definitely true ☐ Mostly true ☐ Don't know ☐ Mostly false ☐ Definitely false

My health is excellent
☐ Definitely true ☐ Mostly true ☐ Don't know ☐ Mostly false ☐ Definitely false

Prospective, open-label, parallel randomized controlled superiority trial comparing a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle versus standard care in sedentary and physically inactive patients with type 2 diabetes

The Italian Diabetes and Exercise Study 2 (IDES-2)



Statistical analysis plan

Final version; 15.09.2012*

Submitted to the Ethics Committee of Sant'Andrea University Hospital
for final approval in the session of 25.09.2012

*Ethics Committee approval and trial registration details added on 12.10.2012

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Table of contents

List of abbreviations

ACR: albumin:creatinine ratio
AE: adverse event
ALT: alanine aminotransferase
AST: aspartate aminotransferase
BMI: body mass index
BP: blood pressure
BUN: blood urea nitrogen
CHD: coronary heart disease
CK: creatine kinase
CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration
CON: control
eGFR**:** estimated glomerular filtration rate
FPG: fasting plasma glucose
γ-GT: γ-glutamyl-transpeptidase
HbA_{1c}: hemoglobin bA_{1c}
HOMA-IR: Homeostasis Model Assessment-Insulin Resistance
HR: heart rate
hs-CRP: high sensitivity-C-reactive protein
IDES_2: Italian Diabetes and Exercise Study 2
INT: intervention
ISF-36: Italian version of the Short Form 36 health survey
MCS: mental component summary measure
PCS: physical component summary measure
LPA: light intensity physical activity
LTPA: leisure-time physical activity
MCV: maximal voluntary contraction
MedDRA: Medical Dictionary for Regulatory Activities
MET: metabolic equivalents
MVPA: moderate-to-vigorous intensity physical activity
NA = not applicable
NYHA: New York Heart Association
PA: physical activity
QoL: quality of life
SAP: statistical analysis plan
SED-time: sedentary time
SF: Short Form 36 health survey
T2DM: Type 2 Diabetes
UKPDS: United Kingdom Prospective Diabetes Study
VO_{2max}: maximal oxygen uptake
WBC: white blood cells
WHO: World Health Organization

1 Trial information

Objective: To compare a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle versus standard care in sedentary and physically inactive patients with type 2 diabetes mellitus (T2DM).

Intervention: Structured counseling consisting of one individual theoretical session, held by a physician (diabetologist), and eight twice-a-week individual theoretical and practical sessions, held by a certified exercise specialist (professionals with a Bachelor degree in exercise science), once a year for three years, on top of standard care.

a. Intervention arm

- Name: Aggregated behavior change techniques.
- Description: one individual theoretical counseling session, held by a physician (diabetologist) plus eight twice-a-week individual theoretical and practical counseling sessions, held by a certified exercise specialist, once a year for three years, on top of standard care.

b. Control arm: general physician recommendations for increasing the amount of daily physical activity (PA) and decreasing sedentary (SED)-time on top of standard care.

Countries of Recruitment: Italy.

Health Condition(s) or Problem(s) Studied: type 2 diabetes mellitus.

Trial duration: 36 months.

EudraCT No.: NA

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.

Acronym: IDES_2.

Registration: ClinicalTrials.gov; NCT01600937; URL https://clinicaltrials.gov/ct2/show/NCT01600937.

Ethical approval: obtained from the Ethics Committee of Sant'Andrea University Hospital on 25.09.2012.

Trial phase: NA.

2 Trial design

The Italian Diabetes and Exercise Study 2 (IDES_2) is a prospective, open-label, parallel, randomized controlled superiority trial comparing a long-term behavioral intervention for adoption and maintenance of a physically active lifestyle versus standard care in sedentary and physically inactive patients with type 2 diabetes.

The flow chart of the study is presented in **Figure 1**.

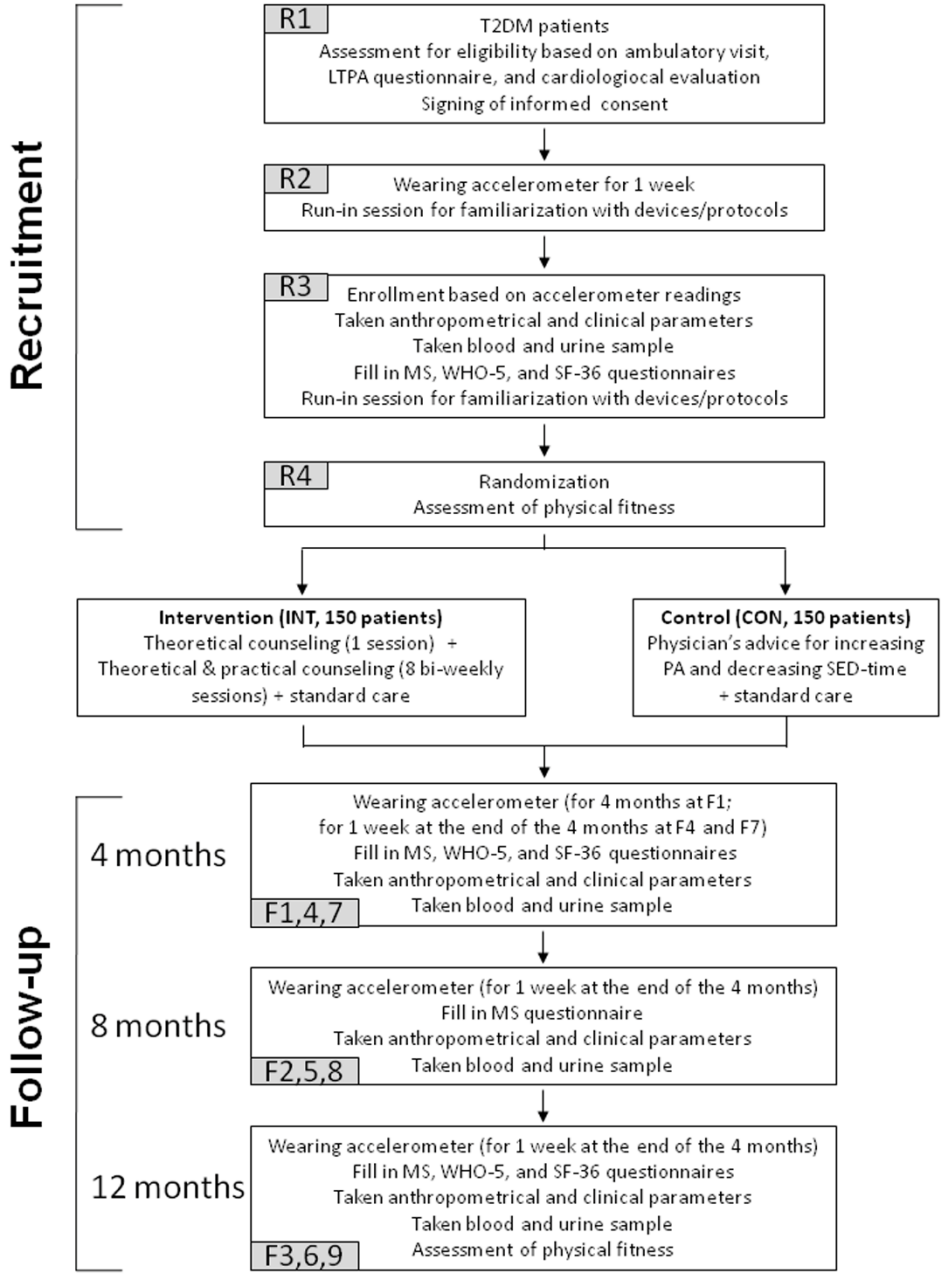

Figure 1. Study flow-chart. Sequence of recruitment and follow-up visits during year 1 (F1-F3), 2 (F4-F6) and 3 (F7-F9) in the IDES_2. T2DM = type 2 diabetes mellitus; LTPA = leisure-time physical activity; MS = musculoskeletal; WHO = World Health Organization; SF = Short Form; INT =intervention; CON = control.

Participants

The main entry criterion is known T2DM (defined by the American Diabetes Association criteria) of at least 1-year duration. Additional requirements are age 40-80 years, body mass index (BMI) 27-40 kg/m^2, sedentary lifestyle (i.e. more than 8 hours/day spent in any waking behavior characterized by an energy expenditure ≤1.5 metabolic equivalents [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, ability to walk 1.6 Km without assistance, and eligibility after cardiologic evaluation.

In order to preserve high internal validity and reduce risk of adverse events, the criteria presented in **Table 1** are used to exclude individuals with conditions limiting or contraindicating PA, affect conduct of the trial, reduce lifespan, and/or affect the safety of intervention.

Table 1. Exclusion criteria. Criteria for excluding patients for entry into the IDES-2. BP = blood pressure; eGFR = estimated glomerular filtration rate; HR = heart rate; NYHA = New York Heart Association.

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

2.1 Objectives and scope of the statistical analysis plan

This statistical analysis plan (SAP) is based on the statistical analyses planned in the trial protocol.

The SAP includes a more detailed procedure for executing the statistical analysis of secondary endpoints, as well as additional supportive analyses of primary and secondary endpoints.

Endpoints

a. Primary: Objectively-measured physical activity and sedentary time.

b. Secondary:

- Physical fitness: cardio-respiratory fitness, muscle strength, flexibility;
- Modifiable cardiovascular risk factors and scores: hemoglobin (Hb) A_{1c}, lipid profile, blood pressure, high-sensitivity C-reactive protein (hs-CRP); United Kingdom Prospective Diabetes Study (UKPDS) total and fatal coronary heart disease (CHD) and stroke 10-year risk scores;
- Musculoskeletal disturbances;
- Well-being/depression;
- Health-related quality of life (QoL).

The primary objective of IDES-2 is to assess the effect of a behavioral intervention versus standard care on the adoption and maintenance of a physically active lifestyle in sedentary and physically inactive patients with type 2 diabetes

Secondary objectives include the assessment of the effect of intervention on physical fitness, modifiable cardiovascular risk factors, musculoskeletal disturbances, well-being/depression, and health related QoL.

Ancillary objectives include the assessment of the percentage of participants achieving pre-specified targets for PA volume (>20 METs-hour·week^{-1}), light intensity (LPA, >6 hours·day^{-1}), moderate-to-vigorous intensity PA (MVPA, $\geq$150 min·week^{-1}), and SED-time (<10 hours·day^{-1}) and the relationship between change in behavior and change in physical fitness, modifiable cardiovascular risk factors, musculoskeletal disturbances, well-being/depression, and health related QoL.

While physicians and exercise specialists will be aware of group assignment, the assessor of the primary outcome (i.e., PA and SED-time from accelerometer readings) will be blinded to group assignment and blinding of blood and urine samples at central laboratory will be achieved using bar codes.

Measurements

Assessment of PA

At screening, the volume of PA will be assessed retrospectively using the Minnesota LTPA questionnaire [46]. At baseline, follow-up and end-of-study, each participant will be outfitted with a uni-axial piezoelectric accelerometer MyWellness Key (Technogym, Gambettola, IT). The advantages of using the MWK accelerometer relies on the fact that it offers the possibilities of storing 30 days of continuous movement detection and provides accurate measures of the minutes spent at light, moderate, and vigorous intensities and the total volume of PA also in individuals with T2DM. The device is attached at the waistband in midline of the right anterior hip and detects the minutes spent at light, moderate and vigorous intensities and the total volume of PA. Each participant will wear the device for 4 months at baseline and for 7 consecutive days every 4 months thereafter. Upon waking (immediately after bathing or showering), participants will be asked to attach the device at the waistband in midline of the right anterior hip and to report on a daily diary the hours spent wearing the instrument, sleeping and napping, and performing non-accelerometer recordable PAs such as swimming, cycling, skiing, etc.

Participants will be asked to wear the device all day (except if swimming) up to bedtime, in order to avoid the influence of the "time accelerometer worn", which may vary from patient to patient. In this way, it will be possible to assume that the time patients are awake without wearing the accelerometer is spent in sedentary activities (e.g. take a shower, get dressed), unless spent in PAs that cannot be performed while wearing the accelerometer (e.g. swimming). Total SED-time, i.e., the daily time the patients are awake without being engaged in a PA, will be then calculated by adding this time to that recorded by the accelerometer with readings <100 counts/min, a threshold which corresponds with sitting, reclining, or lying down, i.e., to <1.5 METs.

Matthews' cut-points will be used to identify time spent in light intensity (100–1951 counts/min corresponding to 1.5–2.9 METs), whereas Freedson's cut-points will be used to determine time spent in PA of moderate intensity (1952–5724 counts/min corresponding to 3–5.9 METs) and of vigorous intensity

(≥5725 counts/min corresponding to ≥6 METs). Time spent in non-accelerometer recordable PAs, as reported on the daily diary, will be added to that recorded by the accelerometer, according to the intensity of each activity. Moderate-intensity PA will be combined with vigorous-intensity PA into MVPAs, as participants spend little time in vigorous-intensity PA.

Volume of PA will be calculated by summing the duration in minutes of PA at each of the three intensities (either recorded by the accelerometer or, for non-accelerometer recordable PAs, derived from the daily diary) multiplied by their average METs intensity. Results will be expressed as METs-hour·week^{-1}.

Assessment of physical fitness

Parameters of physical fitness, i.e., cardio-respiratory fitness, strength and flexibility, will be evaluated at baseline and month 12, 24, and 36 (end-of-study). The tests will be preceded by two consecutive run-in sessions to become familiar with testing devices and protocols.

Cardio-respiratory fitness will be assessed by a maximal treadmill exercise test using a Balke protocol. The treadmill speed will be set at 88 m/min and the grade is 0% during the first minute, 2% during the second minute, then it will be increased every minute by 1%; after 25 minutes, treadmill grade will be kept constant, whereas speed will be increased by 5.4 m/min every minute. Patients will be instructed not to hold the treadmill rails and will be verbally encouraged to reach volitional exhaustion, as reported by the patient or by the physician for medical reasons. The time to exhaustion will be used to estimate maximal oxygen uptake (VO_{2max}), given the high correlation between these two parameters.

For strength assessment, isometric muscle strength will be measured by means of a strain gauge tensiometer (Digimax, Mechatronic GmbH, Germany), as previously described. Isometric tests have been chosen since they are reproducible, do not require complex execution technique, and can therefore be easily performed by both trained and untrained subjects. Upper body muscle strength will be assessed by maximal voluntary contractions (MVCs) performed at a shoulder press (Technogym) along the sagittal plane, with a 90° and 45° angle at the elbow and between the upper arm and the trunk, respectively. Lower limb muscle strength will be assessed by MVCs performed at a costumed leg extension machine (Technogym). The angle at the knee and the hip will be fixed at 90°. These exercise modalities have been chosen in order to test the muscles chains involved in fundamental activities of daily living. In both tests, subjects will be asked to exert their maximal force with both limbs simultaneously and to maintain it for at least 2-3 seconds before relaxing. For each exercise, 3 MVCs will be performed, with 3-min rest interval between contractions. Participants will be asked to perform additional contractions if the MVC of their last attempt exceeds the previous by at least 5 %. During the MVCs, verbal encouragement will be provided always by the same investigator for all subjects.

For hip and trunk flexibility assessment, a standard bending test will be executed. Standing on a step with the legs fully extended, patients will be asked to bend the torso forward to try to touch the ground with their fingertips. The test will be performed three times and the distance between the finger and the ground will be measured by the exercise specialist at the third attempt.

Assessment of modifiable cardiovascular risk factors

Modifiable cardiovascular risk factors will be evaluated at baseline, end-of-study, and every 4 months in-between.

Body weight and height will be measured using scale and stadiometer and BMI will be then calculated as weight (kg)/height (m^2), while waist circumference will be taken at the umbilicus. Body composition will be evaluated by assessing fat mass and fat-free mass by the use of a bioimpedentiometer (Tanita BF664, Vernon Hills, IL, USA). BP will be recorded with a sphygmomanometer after a five-minute rest with the patient seated with the arm at the heart level.

The following biochemical parameters will be measured: HbA_{1c}, fasting plasma glucose (FPG), serum insulin, C-peptide, BMI, waist circumference, body composition, BP, triglycerides, total, LDL and HDL cholesterol, high sensitivity-C-reactive protein (hs-CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transpeptidase (γ-GT), creatine kinase (CK), complete blood count, uric

acid, blood urea nitrogen (BUN), serum creatinine, urinalysis and albumin/creatinine ratio on first-voided urine samples. Biochemical tests will be centralized at the Laboratory of Clinical Chemistry of Sant'Andrea University Hospital, an accredited and ISO9001 certified structure, using standard analytical techniques (**Table 2**).

Table 2. Laboratory tests. Biochemical measurements in the IDES-2. IDES = Italian Diabetes and Exercise Study; HbA_{1c} = hemoglobin A_{1c}; HPLC = high-performance liquid chromatography; FPG = fasting plasma glucose; hs-CRP= high sensitivity-C-reactive protein; AST = aspartate aminotransferase; ALT = alanine aminotransferase; γ-GT = γ-glutamyl-transpeptidase; CK = creatine kinase; BUN = blood urea nitrogen.

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
Insulin	Chemiluminiscent immunometric assays (Immulite 2000 Thes)	Diagnostic Products Corporation, Los Angeles, CA, USA
C-peptide	Chemiluminiscent immunometric assays (Immulite 2000 Thes)	Diagnostic Products Corporation, Los Angeles, CA, 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
LDL 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
AST	VITROS 5,1 FS Chemistry System	Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA
ALT	VITROS 5,1 FS Chemistry System	Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA
γ-GT	VITROS 5,1 FS Chemistry System	Ortho-Clinical Diagnostics Inc, Raritan, NJ, USA
CK	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
BUN	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

Then, the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index will be calculated from FPG and insulin levels, whereas glomerular filtration rate (GFR) will be estimated from serum creatinine by the use of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Global and fatal CHD

and stroke 10-year risk scores will be calculated using the United Kingdom Prospective Diabetes Study (UKPDS) risk engine.

Assessment of musculoskeletal disturbances

Musculoskeletal symptoms are evaluated by the use of a 50-item self-report questionnaire, which investigates shoulder, arm, elbow, wrist, hand, spine, hip, knee, ankle, and foot problems.

Assessment of well-being/depression

Screening for depression is performed by asking participants to fill in the World Health Organization (WHO)-5 Well-being Index, a five-item questionnaire, which was found to be suitable for use in outpatients with type 1 and T2DM.

Assessment of health-related QoL

Health-related QoL is evaluated using the Italian version of the SF-36 health survey (ISF-36), which yields four physical and four mental health component scores and has been previously validated in subjects with T2DM. The physical and mental component summary measures (PCS and MCS, respectively) are normalized to a population mean of 50 and SD of 10.

Adverse events

Low-to-moderate risks are associated with PA/exercise performed during the theoretical and practical counseling sessions and assessment of physical fitness.

Risk of musculoskeletal injuries is minimized as much as possible through supervision by a certified exercise specialist. Risk of other adverse events including cardiovascular events is minimized by thorough cardiological examination before enrollment.

Adverse events are reported at intermediate visits and, for INT subjects, also at supervised sessions, by completing a standard form.

3 Evaluability of subjects for analysis

The following analysis sets are defined:

Full analysis set (FAS): all randomized subjects. The statistical evaluation of the FAS will follow the intention-to-treat (ITT) principle, and subjects will contribute to the evaluation ‘as randomized’.

Per protocol analysis (PPA): A per-protocol analysis, restricted to subjects followed for the entire study duration.

4 Statistical considerations

4.1 General considerations

No interim analyses or other analyses of between-group data will be performed before the database is locked.

Missing values will not be replaced by imputed values.

Statistical significance will be declared when the p-value is found to be less than 0.050. Statistical tests will be two-tailed.

4.2 Sample size calculation

Sample size calculation is based on preliminary accelerometer data showing that daily PA in sedentary, physically inactive T2DM patients is 10.5±4.1 METs·h^{-1}·wk^{-1}.

To observe a 15% increase in daily PA with a statistical power of 90% (α=0.05) by unpaired t-test, 142 patients per arm are needed (284 total). A sample size of 300 patients will allow supporting a 5% dropout rate, as observed in the IDES.

4.3 Statistical methods

4.3.1 Analysis of the primary and secondary endpoints

The χ^2 test or, where appropriate, 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 for primary and secondary endpoints will be applied to all randomized patients with a baseline and at least one post-baseline value. The superiority of the intervention versus standard care on the primary and secondary endpoints will be assessed by a repeated measures analysis of covariance (ANCOVA - using PROC MIXED in SAS software) of change from baseline to the end-of-study. In case of differences between groups in baseline values, we will consider a baseline adjustment to improve power.

The effect of the intervention on the primary and secondary endpoints will be analyzed both short-term, after the initial 4 months, during which the participants will wear the accelerometer continuously, and long-term, at the end of the 3-year period.

To account for differences in change medication throughout the study period between the two groups, which might affect performance with respect to cardiovascular risk factors, both multiple regression and sensitivity analyses will be perform. 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, use adjustment methods for missing data, and conduct a sensitivity analysis to assess the robustness of inferences about treatment effects to various missing-data assumptions (1). In particular, repeated measures models with an autoregressive correlation type matrix will be applied in order to account for both missingness at random and potential correlation within subjects. These methods are more efficient in that they allow evaluating all subjects, even those with incomplete data (1).

To guarantee replicability and avoid outcome selective reporting, a fully specified statistical analysis plan will be written before analysis.

Statistical analyses will be performed by at the Department of Clinical Pharmacology and Epidemiology, Consorzio Mario Negri Sud, S. Maria Imbaro, Italy, using SAS software release 9.3 (Cary, NC, USA).

Reference

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

4.3.2 Exploratory analysis of subgroups

Exploratory analysis of the primary and secondary endpoints will be performed in several population subgroups. The effect of the following subgroups (main effect and interaction with treatment) will be explored separately by adding them to the original model:

- age: <65 years versus ≥65 years;
- gender: males versus females;
- treatment: non-insulin versus insulin;
- eventually, baseline, end-of-study, or baseline-to end-of study strata of any of the measured variables.

4.3.3 Adverse events

Safety analyses will be conducted on the safety population. The number of adverse events (AEs) will be analyzed using Poisson regression model with correction for overdispersion. Frequency distributions by treatment group of numbers of patients, number of episodes, and incidence rates (number of events per patient-year) of hypoglycemic events will be presented; incidence rates will be compared between groups and reported as incidence rate ratio with 95% CI.

AEs will be mapped to the Medical Dictionary for Regulatory Activities (MedDRA). The newest version of the dictionary available at the time of database lock will be the version used.

The summaries of AEs display the number and percentage of subjects with at least one event. Proportion of participants experiencing AEs will be compared using the χ^2 test, or Fisher exact test, where appropriate.

4.4 Risk of bias

Several methods are used to minimize risk of any type of bias (**Table 3**).

Table 3. Methods against bias. List of actions taken to minimize risk of bias.

Type of bias	Action(s)
Selection bias	Random sequence generation Allocation concealment
Performance bias	Objective target-driven regulation of glucose-, lipid- and BP-lowering treatment by physicians
Detection bias	Blinding of assessors of primary outcome (i.e., PA and SED-time from accelerometer readings) and blood and urine testing.
Attrition bias	Keep up to date contact information for contacting participants at regular intervals Collect complete data for both the primary and secondary outcomes, regardless of whether subjects continue to receive treatment
Reporting bias	Preparation of a fully specified statistical analysis plan before analysis

5 Format of tables/listings

Table A. Baseline features of the whole cohort and by study arm.

Variable	Total	CON	INT	*P* INT vs CON*
N				
PA volume, METs-hour·week^{-1}				
LPA, hours·day^{-1}				
MVPA, min·day^{-1}				
SED-time, hours·day^{-1}				
VO_{2max}, ml·min^{-1}·kg^{-1}				
Upper body strength, Nm				
Lower body strength, Nm				
Bending, cm				
Age, years				
Male gender, n (%)				
Smoking, n (%)				
Never				
Former				
Current				
Diabetes duration, years				
HbA_{1c}, %				
FPG, mg·dl^{-1}				
Insulin, µU·ml^{-1}				
C-peptide, ng·ml^{-1}				
HOMA-IR				
Body weight, kg				
BMI, kg·m^{-2}				
Fat mass, %				
Fat-free mass, kg				
Waist circumference, cm				
Triglycerides, mg·dl^{-1}				
Total cholesterol, mg·dl^{-1}				
HDL cholesterol, mg·dl^{-1}				
LDL cholesterol, mg·dl^{-1}				
Systolic BP, mmHg				
Diastolic BP, mmHg				
hs-CRP, mg·dl^{-1}				
WBC, n·10^{9}·l^{-1}				
AST, U·l^{-1}				
ALT, U·l^{-1}				
γ-GT, U·l^{-1}				

CK, $U \cdot l^{-1}$
Uric acid, $mg \cdot dl^{-1}$
BUN, $mg \cdot dl^{-1}$
Serum creatinine, $mg \cdot dl^{-1}$
eGFR, $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$
ACR, $mg \cdot g^{-1}$
UKPDS CHD 10-year risk score
UKPDS fatal CHD 10-year risk score
UKPDS stroke 10-year risk score
UKPDS fatal stroke 10-year risk score

Values are mean±SD or number of cases (percentage). * Student t-test for normally distributed continuous variables, Mann-Whitney U-test for non-normally distributed continuous variables, χ^2 test for categorical variables. CON = control group; INT = intervention group; PA = physical activity; METs = metabolic equivalents; LPA = light intensity PA; MVPA = moderate-to-vigorous intensity PA; SED-time = sedentary time; VO_{2max} = maximal oxygen uptake; HbA_{1c} = hemoglobin bA_{1c}; FPG = fasting plasma glucose; HOMA-IR = Homeostasis Model Assessment-Insulin Resistance; BP = blood pressure; hs-CRP = high sensitivity-C-reactive protein; WBC = white blood cells; AST = aspartate aminotransferase; ALT = alanine aminotransferase;, γ-GT = γ-glutamyl-transpeptidase; CK = creatine kinase; BUN = blood urea nitrogen; eGFR = estimated glomerular filtration rate; ACR = albumin:creatinine ratio; UKPDS = United Kingdom Prospective Diabetes Study; CHD = coronary heart disease.

Table B. Change from baseline to end-of-study in primary and secondary outcomes in INT vs CON participants.

	CON		INT		INT vs CON	
	Change end-of-year 3 vs baseline (95% CI)	***P***	**Change end-of-year 3 vs baseline (95% CI)**	***P***	**Mean difference (95% CI)**	***P***
Primary outcomes						
PA volume, METS-hour·week^{-1}						
LPA, hours·day^{-1}						
MVPA, min·day^{-1}						
SED-time, hours·day^{-1}						
Secondary outcomes						
VO_{2max}, ml·min^{-1}·kg^{-1}						
Upper body strength, Nm						
Lower body strength, Nm						
Bending, cm						
HbA_{1c}, %						
FPG, mg·dl^{-1}						
Body weight, kg						
Fat mass, %						
Fat-free mass, kg						
Waist circumference, cm						
Triglycerides, mg·dl^{-1}						
Total cholesterol, mg·dl^{-1}						
HDL cholesterol, mg·dl^{-1}						
LDL cholesterol, mg·dl^{-1}						
Systolic BP, mmHg						
Diastolic BP, mmHg						
hs-CRP, mg·dl^{-1}						
eGFR, ml·min^{-1}·1.73 m^{-2}						
ACR, mg·g^{-1}						
UKPDS CHD 10-year risk score						
UKPDS fatal CHD 10-year risk score						
UKPDS stroke 10-year risk score						
UKPDS fatal stroke 10-year risk score						

CON = control group; INT = intervention group; CI = confidence interval; PA = physical activity; METs = metabolic equivalents; LPA = light intensity PA; MVPA = moderate-to-vigorous intensity PA; SED-time = sedentary time; VO_{2max} = maximal oxygen uptake; HbA_{1c} = hemoglobin A_{1c}; FPG = fasting plasma glucose; BP = blood pressure; hs-CRP = high sensitivity-C-reactive protein; eGFR = estimated glomerular filtration rate; ACR = albumin:creatinine ratio; UKPDS = United Kingdom Prospective Diabetes Study; CHD = coronary heart disease.

Table C. Adverse events in the whole cohort and by study arm.

	Total	CONT	INT	*P* INT vs CON*
Outside theoretical and practical sessions				
Elective surgery				
Surgery 1				
Surgery 2				
Surgery 3				
Surgery 4				
Surgery 5				
Surgery 6				
Surgery…				
Medical conditions				
Medical condition 1				
Medical condition 2				
Medical condition 3				
Medical condition 4				
Medical condition 5				
Medical condition 6				
Medical condition….				
Deaths				
From cardiovascular disease				
From cancer				
From…				
Total				
During theoretical and practical sessions				
Severe hypoglycemic episode		NA		NA
Mild hypoglycemic episode		NA		NA
Tachycardia/arrhythmia		NA		NA
Musculoskeletal injury/discomfort		NA		NA
…		NA		NA
Total				

Values are number of cases. CON = control group; INT = intervention group; NA = not applicable. χ^2 test or, where appropriate, Fisher's exact test, for between-group comparison of frequencies of adverse events.