Strength training as a supplemental therapy for androgen deficiency of the aging male (ADAM): Study protocol for a three-arm clinical trial.

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Aims

The overall aim of the trial is to examine the effect of a 12-week strength training program with and without TRT on body composition, physical function, selected biochemical markers of metabolic health, histological and molecular parameters and the quality of life of patients with ADAM.

Study design

The study is a clinical trial with three arms comparing the effect of strength training with testosterone replacement therapy (ST + TRT), strength training alone (ST) on hypogonadal males and on a control group of healthy eugonadal males (HM), also engaged in strength training for 12 weeks.

Trial status

At the time of the first submission of the protocol, the trial was in the phase of participant recruitment. The recruitment began in February 2017 and the last part of data collection is expected to end in August 2019.

Participants

Subjects will be included from urological units at Department of Urology, University Hospital-Petrzalka, Bratislava, Slovakia; Department of Urology, Faculty of Medicine, Comenius University, Bratislava, Slovakia and 5. Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia. The study will involve in total sixty-six male participants divided into three groups (n = 66): group 1, males with hypogonadism who are undergoing testosterone replacement therapy (TRT) (n=22); group 2, newly diagnosed males with hypogonadism without testosterone replacement therapy (NON-TRT) (n=22); group 3, healthy eugonadal men (HM) (n=22). The participants from all groups engaged in strength training. The volunteers are screened for testosterone levels before the start of the participation by the specialist.

The most important inclusion criteria for participation in the study from the patient population are age 40-60 years old, subjects with hypogonadism on TRT or newly diagnosed patients of hypogonadism. The hypogonadal patients fulfilling the criteria for study participation will be verified for low testosterone before entering the study. The same verification will take place at the end of the study. The most important exclusion criteria include regular strength training, conditions that are medical contraindications and prostate cancer or

abnormal serum PSA levels without adverse histological examination. All inclusion and exclusion criteria are listed in Additional file 1. In addition to written information, eligible subjects will be verbally informed about the study by their responsible urologist and the study officials before participation. TRT provided to patients is intramuscular (IM) injection of testosterone undecanoate (TU) at a dose of 1000 mg repeated every 12 weeks. Testosterone undecanoate (Nebido) is the only injectable form of testosterone used at the institutes of collaborating physicians of the study. According to our knowledge, this form of T at dose of 1000 mg is the most stable of all available preparations for 3 months' period, which is considered a standard treatment in Slovakia. Shorter-acting forms may cause more pronounced fluctuations in 24-hour circulating levels of testosterone. The participants will be asked to not change their habitual dietary intake and physical activity patterns. Participants will be asked to continue in physical activities as before, but any kind of regular physical activity, especially strength training or any other kind of weight training during the intervention will be also prohibited. The exclusion criteria reject any participant, who performed any kind of regular strength training one year prior to study.

Strength training intervention

The strength training protocol will be a modified strength exercise program from Segal et al. [61] which was used in similar group of patients. The participants will perform 24 training sessions of strength training protocol with the frequency of two training sessions per week for 12 weeks. There will be at least 48 hours rest period between two subsequent training sessions (Monday and Thursday). The intervention will take place at the Faculty of Physical Education and Sport, Comenius University in Bratislava, Slovakia. All training sessions will be supervised and guided by professionals with university degree in sports training to ensure safety, correct technique and progression in training load, with a maximum of three participants per one trainer. The participants will be familiarised with the equipment and exercise technique one week before the start of the intervention. The technique corrections will be possible during the whole intervention if needed. Ten repetition maximum (RM) and 12RM diagnostic test for all exercises will be conducted during the first week of training intervention.

Each training session will include a 5-minute dynamic warm-up, consist of 10 exercises for approximately 30 seconds of each, and exercises will be focused on main muscle groups (Table 2).

Table 2: Dynamic warm-up

Dynamic warm-up exercises	Number of repetitions
Walking low skip	8 times each leg
Walking high knee skip	8 times each leg
Walking knee to chest	8 times each leg
Walking hamstring stretch	6 times each leg
Walking lunge	6 times each leg
Standing lateral lunge	6 times each leg
Egyptian mobility exercise	6 times each arm
External rotation exercise	6 times each arm
Hip hinge exercise	8 times
Air squat	8 times

The strength protocol exercises will be performed with free weights and on machines. The training program consist of six exercises for upper and lower body at an intensity of 60-80% (8 – 12RM: the load that induces technique failure in eight or twelve repetitions) of one-repetition maximum and takes approximately 60 minutes. The inability to perform full repetition will be assessed by a supervisor or by participants' feedback. The participants will be instructed to perform a concentric action for 2 seconds and immediately after an eccentric action also for 2 s. There will be 90 seconds rest period after each set. The same duration rest period will be between all of the exercises. The rest periods will be controlled by timer (The miniMAX, Gymboss, USA). The load will be added, if participant can complete prescribed number of repetition in each set of the exercise. More detailed strength training protocol can be seen in Table 3. During the first three weeks of the intervention, there will be one set in the beginning with light weight to focus on safety and technique. After that three more sets with weight close to 60 – 80 % of 1RM will follow. After first three weeks, the number of sets will be increased to four.

Table 3: Strength training protocol

Week	Number of exercises	Number of sets	Number of repetitions	Resistance	Rest period	Tempo

1 – 3. week	3+3 (UB, LB)	3	10-12	10-12RM	90s	2:0:2:1
4 – 6. week	3+3 (UB, LB)	4	10-12	10-12RM	90s	2:0:2:1
7– 9. week	3+3 (UB, LB)	4	6-8	6-8RM	90s	2:0:2:1
10 – 12. week	3+3 (UB, LB)	4	6-8	6-8RM	90s	2:0:2:1

UB – upper body, LB – lower body, RM – repetition maximum, Tempo – duration in seconds during the repetition - 2s (eccentric): 0s (end range of the motion): 2s (concentric): 1s (rest between repetitions in the starting position)

The exercises performed during every session will be: leg press, split squat, bench press. The exercises alternating through the week are knee extension with leg curl, seated row with seated pull down and incline dumbbell bench press (training equipment provided by KOHI Leopoldov, Slovakia and Technogym, Italia). Since unilateral exercises (e.g. one leg squats) develop similar magnitude of muscle activity with producing less load on the spine, thus they are safer [62], the split squats are chosen instead of regular squats. Prescribed exercises in the strength training protocol for every training sessions can be found in Table 4. Each session will be supervised by at least two professionals, who received strength training programme and record every repetition and set made in each session in an individual training plan. At the start of each session, the trainers will ask participants if they experienced any adverse events since the last session and record reported events. All adverse events during the training session will be written down into paper spread sheet and processed afterwards. Each training session will be monitored with an attendance list, with minimum 85% attendance during the study. Each session will be marked as successfully completed when at least 80% from the total volume and intensity of the training protocol planned for the particular training session is performed. If a participant will be unable to perform any of the exercises or sets, this will be recorded into a prepared training plan and the situation will be managed during the first week during familiarization with the training protocol. The appropriate alternative exercise will be considered depending on the restriction or participant's limitation.

Table 4: Training sessions, type of exercises and type of resistance

1st training session	Type of resistance	2nd training session	Type of resistance
Split squat	Dumbbells	Bench press	Barbell
Bench press	Barbell	Split squat	Dumbbells
Leg press	Machine	Incline press	Dumbbells
Seated row	Machine	Leg press	Machine
Leg curl	Machine	Pull down	Machine
Lateral raise	Dumbbells	Knee extension	Machine

Clinical outcomes

Clinical outcomes will be collected one week before the intervention (pre-intervention assessments) and one week after the intervention (post-training assessments). All outcomes, specific variables and assessments in each testing are listed in Additional file 2. All participants will be tested at the same time of the day, and asked to avoid caffeinated and alcohol beverages before the assessments.

Familiarization

To secure validity of the physical tests, all subjects undergo a session of familiarization 7 days prior to the intervention assessments. All sessions are performed based on the same guidelines, but after the familiarization session the load of each resistance exercise will be adjusted to match the expected maximum.

Primary outcome measure

Lean mass (LM)

The primary outcome of the study will be the change in lean mass (LM) measured by Dual-energy X-ray Absorptiometry (Hologic fan-beam bone densitometer Discovery QDR series). The changes in lower and upper body LM are analysed separately because of differences in androgen sensitivity in leg muscles compared to neck, chest and shoulder muscles [63]. Due to very similar results but greater participant comfort [63] we decided to use The National Health and Nutrition Examination Survey (NHANES) protocol, which required the participant to be positioned in a supine position in the middle of the densitometry table with head straight, space between the arms and torso, palms flat on the table, and feet together secured by a strap. The

systematic review of Shiel et al. [64] showed a strong level of agreement as illustrated by high ICC's and CCC's between the Nana and NHANES positioning protocols, however systematic bias within limit of agreement plot and a large difference in 95% confidence limits indicates that the protocols should not be interchanged when assessing an individual.

Secondary outcome measures

Body composition

Other body composition parameters (fat mass, total body mass) will be measured at the same time so also the protocol is the same as with the primary outcome. The height will be measured by stadiometer and waist circumference will be measured by stretch-resistant tape that provides a constant 100 g tension. The body mass index is afterwards calculated and reported.

Muscle strength

Muscle strength of lower extremities will be measured as force production during maximal voluntary contraction (MVC) isometric knee extension and isometric knee flexion knee dynamometer (ARS dynamometry, S2P ltd., Ljubljana, Slovenia). Each of the test will be performed 6 time with three practise trials and three recorded trials. For the first practise trial, participants will be instructed to achieve approximately 50% of the maximum, with 20 seconds rest period. The second and third practise trial will be performed at 80% of the maximum with 20 second rest periods. The last three trials will be performed with maximal voluntary effort and will be recorded. The best out of three will be taken for further analyses. Rest period during recorded trials will be 60 seconds. During the MVC, the participants will be asked to push/pull as strong as possible and hold for five seconds. Intra-session repeatability for MVC is the 5.7 CV % and 0,98 ICC. Additionally, with awareness of health issues (such as higher blood pressure) and because of a safety reasons, dynamic leg press 1RM (one repetition maximum) will be predicted from multiple repetition maximum testing [65].

For assessing the muscle strength of the upper extremities, the isometric MVC handgrip strength will be measured by Camry Digital Hand Dynamometer. The participant will stand upright and holds the dynamometer in the hand next to the body, with the minimal or none flexion in the elbow joint. The base of the handle will be on the first metacarpal, while the handle should rest on the middle of the four ringers. None of the body parts will be allowed to move. The test will be performed with three practise trials. First on 50% and the others on 80%

of their perceived maximum with 20 seconds' rest period. After that, three maximum trials with rest period of 60 seconds will be recorded and the best out of three will be taken for further analyses. The participants will be encouraged to give their maximum effort. The participant will squeeze the dynamometer for 5 seconds. After the test with dominant hand, the test will be performed for non-dominant hand.

Cardio-respiratory fitness

Cardio-respiratory fitness will be measured by The Single Stage Treadmill Walking Test [66], where the participants will be asked to walk on Pro Treadmill (Woodway, USA). During the walking test, participants will wear same shoes they will use during the whole intervention. The speed during the test can be changed if needed. The procedure will be performed once and heartbeat will be tracked by heart rate monitor attached on the chest. VO_2max will be calculated according the literature [66].

10-m preferred walk-speed and 10-m maximum walk-speed will be measured by timing gates WITTY GATE (MicroGate, Italy). Participants will walk 10 meters and the time will be measured for the intermediate 6 meters. This allow acceleration and deceleration. The gates will be placed on 2-meter mark and 8-meter mark. The timing starts when participant cross the first mark and stop when the 8-meter mark is crossed. There will be three trials for preferred and three trials for maximum walk-speed. The outcome measure will be velocity in meters per second calculated as mean of the three trials or the best trial from the preferred and maximum walk-speed test, respectively. Participants will be asked to perform at preferred walking speed first followedand then at the fastest walking speed possible.

Psycho-social functioning

The general health status will be measured by The Short Form (36) Health Survey patient-reported survey of patient health (SF-36). In addition, clinically investigating the health-related quality of life (HRQoL) symptoms of aging men are measured by Aging Males' Symptom (AMS) Scale. The AMS scale had internal consistency [$\alpha = 0.89$ (95% CI 0.88-0.90)]; the mean alpha estimates across the AMS subscales ranged from 0.79 to 0.82. The AMS scale also had good test-retest reliability [$r = 0.85$ (95% CI 0.82-0.88]; the test-retest reliability coefficients of the AMS subscales ranged from 0.76 to 0.83 [67]. AMS is a standardized scale according to psychometric norms. Most of the currently available language versions were

translated following international standards for linguistic and cultural translation of quality of life scales. [68].

Serological outcomes

Fasting morning venous blood will be taken after overnight (10-hour) fasting and 15 min rest from cubital vein from 8:00 am to 10:00 am [69] into closed system collection tubes containing beads coated with a clotting activator and polyacryl ester-gel (Sarstedt AG & Co, Germany). The blood will be centrifuged (3000g, 4°C, 10min) immediately after sampling to obtain EDTA plasma or they will be centrifuged (3000g, 4°C, 20min) after 30min at RT, to obtain serum. The haematological and biochemical parameters analyzed immediately will be haemoglobin, hematocrit, leucocytes, thrombocytes, glucose, urea, sodium, potassium, calcium, ALAT, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, testosterone, oestrogen, LH, FSH, SHBG, albumin, bilirubin, total protein, CRP, insulin and PSA. Plasma and serum aliquots (500 ul) will be stored at -20°C (analysis within 6 months) and at -80°C for the long term storage. Bioactive molecules (myokines, exerkines, released from skeletal muscle and/or other tissues) which could be associated with the adaptive response to exercise in all patients will be quantified.

Muscle cellular outcomes

Muscle biopsies will be obtained from approximately 80% of the subjects included in the study. Subjects not willing to undergo biopsy are still eligible for trial participation.

With the subject in a supine position, a 5 mm Muscle Biopsy Cannula (Bergstrom-Stille, Sweden) with manual suction is used to obtain muscle samples (200 mg), under local anaesthesia (Lidocain 2%). Before the intervention, the biopsy will be obtained from the mid-section of the right m. vastus lateralis, and after the intervention the biopsy will be obtained 3 cm proximal to the pre-intervention biopsy.

Muscle fibre size and regulators of muscle fibre size

Muscle fiber size, measured as muscle fiber cross sectional area, represents the primary muscle cellular outcome. Secondary muscle cellular outcomes reflecting regulators of muscle fibre size

are a) number of myonuclei per muscle fiber b) number of satellite cells per muscle fiber, c) number of satellite cells and myonuclei positive for androgen receptors and d) proteins involved in muscle protein degradation (muscle breakdown). The number of satellite cells will be quantified on frozen muscle cross sections with a immunohistochemical protocol as described in Bjornsen et al. [70] (Pax7 + Laminin + DAPI).

Muscle fibre cross sectional area and regulators of muscle fibre size are analysed by immunohistochemistry on cross sections of muscle biopsies and by western blots and enzyme-linked immunosorbent assay (ELISA) in muscle homogenate.

Muscle fibre cross sectional area is measured by cutting transverse serial sections of the muscle biopsy (8 μm thick) with a cryostat microtome (Microm, Germany) at -22°C and mounted on glass slides. Serial sections are immunohistochemically stained for fibre types (type I and type II) (used to measure muscle fibre cross sectional area), number of satellite cells, number of myonuclei and number of satellite cells and myonuclei positive for androgen receptors. Muscle fibre cross sectional area is measured for the different fibre types separately.

Statistical Analysis

Normality of the data distribution will be assessed by comparing histogram of the sample data to a normal probability curve and outliers will be identified as values distant for more than 3σ from the average. Normality will be further tested with Kolmogorov-Smirnov test if needed.
Differences between normally distributed variables will be evaluated by the Analysis of variance with repeated measures and Bonferroni post-hoc test, differences between pre- and post-training values of the specific subpopulation will be evaluated with a paired Student's t-test. Non-normally distributed variables will be log transformed. Variables that could not be log transformed to normal distribution will be tested with non-parametric tests (Mann-Whitney test and Wilcoxon rank test).
Cohen's d will be used to calculate effect size (ES), represented by 'd' and interpreted as < 0.2 is a small, 0.2-0.8 is a moderate, and > 0.8 is a large effect size.
For studying the relationships between the various outcomes, the Pearson or Spearman correlation tests will be used.
All statistics were performed using a statistical software Statistical Package for the Social Sciences (SPSS) 21.0 (IBM Inc., Armonk, New York, U.S.,) and p values < 0.05 will be

considered significant. Data will be presented as means and standard deviations. Missing endpoint data will disqualify patient from the endpoint analysis. Missing single value, of training progression records will be replaced by the last observed value.

Background variables

Information about medical situation as time points for treatment and stage of symptoms are collected from the medical record. Past illnesses and other medical problems are also reported in the questionnaire.

Patients and public involvement

Patients (study participants) will be informed about the individual results of the baseline examination as well as on the primary and secondary outcomes of the study, in a form of individual consultation with a research team member.

Patients or public (patient organizations) were not involved in the development of the research question or study design. However, they will be asked to help with recruitment, and will also be involved in the conduct of the study with the power to shape (individualize) the training intervention according to individual preferences, prior experiences and medical conditions. Moreover, they will be involved in individualizing the follow-up intervention protocol, shaping thus the long-term exercise programme to increase its sustainability.

Sample size

The pre-existing data from our previous 12-week exercise intervention study related to fat (5.6% decrease $p=0.002$) & lean body mass (1.8% increase, $p=0.047$, DXA) and that of maximal voluntary contraction force measured on linear leg-press (31% increase, $p<0.0001$, 1RM). Lean body mass was used to determine sample size for the population of the designed intervention study. The Type I error probability was set at 0.05 and the power to 0.90 and 0.95. Results indicate that 22 patients per group will be sufficient to detect exercise intervention related change of 1,06 ± 1,56 kg (average ± SD) of lean body mass at the power of 0.90, accounting for the 10% patients drop-out.

Ethics and Dissemination

This trial was approved by Ethics Committee of the University Hospital in Bratislava, Slovakia (ref. trial number: 127/2017). All the participants will be fully informed on the study protocol risks and benefits and will provide the written informed consent prior entering the study. Participation in the trial is fully voluntary. Inability to comply with the study protocol will not affect the healthcare. Data will be stored and handled anonymously using the coding system complying with the General Data Protection Regulation 2016/679. All unexpected, serious adverse events will be reported to the study sponsor as well as to the relevant health insurance company within 7 days. The findings of this trial will be published in peer review journals, scientific conferences with main audience of healthcare professionals, healthcare providers, but also patients and their families. Trial was registered at ClinicalTrials.gov: NCT03282682.

AUTHORS' CONTRIBUTIONS

MK, JC, TR, MS participated in the study design and drafted the manuscript, GB participated in the development of the intervention protocol, TR, BU and JU designed protocol for biological sample collection and processing, and will participate in biological material sampling & analyses. MP, ZK, JP, BK and PB provide access to patients. MK, MS, JC, JU and MK performed data analysis. All authors contributed to and approved the present manuscript.

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Competing interests statement

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