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Strength training as a supplemental therapy of androgen deficiency of the aging male (ADAM): rationale and design

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Abstract

Introduction: Androgen deficiency of the aging male (ADAM) is a clinical syndrome resulting from the low production of androgens (testosterone levels <6.9 nmol/l) with symptoms including decline in lean mass, muscle strength, increases in body mass and overall fat mass. The aim of the study is to examine the effect of a 12-week strength training program on body composition, physical function, and selected biochemical markers of metabolic health in patients with ADAM.

Methods and analysis: The study is 3-group controlled 12-week experiment to assess the effect of strength training (ST) on hypogonadal patients with testosterone replacement therapy (TRT) and newly diagnosed males without TRT (NON-TRT). Age matched healthy eugonadal males (control group) is also engaged in strength training. Clinical and muscle cellular outcomes are collected before the end of intervention (pre-intervention assessments) and after the intervention (post-intervention assessments). Post-intervention measurements start 7 days and go up to 3 weeks after intervention. Clinical outcomes are body composition (lean mass, fat mass, bone mineral density and total body mass) measured by Dual-energy X-ray Absorptiometry, physical functioning (muscle strength, cardio-respiratory fitness) assessed by physical tests and psycho-social functioning (health related quality of life, Aging Males' Symptom scale). The most important haematological and biochemical parameters included are glucose, total cholesterol, LDL cholesterol, HDL cholesterol, testosterone, LH, FSH, SHBG, total protein, CRP, insulin and PSA. Muscle cellular outcomes are muscle fiber size, regulators of muscle fiber size and regulators of muscle fiber function. Muscle cellular outcomes are measured on muscle cross sections and muscle homogenate from muscle biopsies obtained from *m. vastus lateralis*.

Strengths and limitations of this study

- **There is very limited number of articles published in topic of male hypogonadism and effect of physical activity to well-being and body composition and other parameters of training adaptation.**

- **The above-mentioned studies did not focus on possible physiological and metabolic mechanisms responsible for the positive effects of resistance training at circulating, cellular and molecular level.**

- **Up to date, there are no studies investigating the effects of strength training on the regulation of muscle mass and neuromuscular function on a cellular level in male hypogonadal patients.**

- **One of the goals is to propose strength training as a potential supplemental therapy to fight against adverse effects of male hypogonadism.**

Introduction

Testosterone is one of the most potent naturally secreted androgenic-anabolic hormone, and its biological effects include, among others, promotion of skeletal muscle growth [1]. Testosterone stimulates protein synthesis (anabolic effect), inhibits protein degradation (anti-catabolic effect) and these effects account for the promotion of muscle hypertrophy by testosterone [2]. Some other important physiological effects of testosterone in male adults are maintaining reproductive tissues, stimulating spermatogenesis, and sexual functions, increases in nitrogen retention and in lean body mass, maintaining bone mass, promoting sebum production, and axillary and body hair growth, and stimulation of erythropoiesis [3].

Aging beyond 35–40 years is associated with a decline of 1–3% per year in circulating testosterone concentration (1.6% in total and 2–3% in bioavailable testosterone) in men. This reduction can eventually lead to very low resting concentrations of circulating testosterone, a condition that has been termed andropause [4-6].

The Endocrine Society recommends 10.4 nmol／l as the lower limit of normal total testosterone. The American Association of Clinical Endocrinologists suggested 6.9 nmol／l and the International Society of Andrology, International Society for the Study of Ageing Male, European Association of Urology, European Academy of Andrology, American Society of Andrology recommendations suggest that 8 nmol／l is a limit below which patients can be considered as hypogonadal and will usually benefit from testosterone replacement treatment. The Endocrine Society defines male hypogonadism as a clinical syndrome that results from failure of the testis to produce physiological levels of testosterone (androgen deficiency) and normal number of spermatozoa. Hypogonadism is caused by disruption of one or more levels of the hypothalamic–pituitary–gonadal axis [7]. All the causes of male hypogonadism can be found in Table 1.

Primary hypogonadism

Primary hypogonadism is caused by testicular failure and is characterised by low serum testosterone and high LH and FSH concentrations. For this reason, primary hypogonadism is also known as hypergonadotropic hypogonadism. Primary hypogonadism can result from testicular injury, tumour, or infection; genetic defects affecting testicular development (e.g. Klinefelter syndrome), as well as chemotherapy, radiation treatment or alcohol abuse [8-9].

Secondary hypogonadism

In secondary hypogonadism (hypogonadotropic hypogonadism), defects in the hypothalamus or pituitary result in low testosterone levels because of insufficient stimulation of the Leydig cells. It is also associated with low or low-normal FSH and LH levels. Patients with secondary hypogonadism can have their fertility restored by suitable hormonal stimulation, whereas those with primary hypogonadism resulting from testicular failure cannot. Secondary hypogonadism can be caused by number of conditions including hypothalamic and pituitary disorders or lesions, hyperprolactinemia and Kallmann syndrome (which causes a GnRH deficiency) [9]. Certain medications and illnesses can also affect the hypothalamic–pituitary system resulting in hypogonadism [10].

Table 1: Causes of male hypogonadism [8,9,52]

Primary hypogonadism	**Secondary hypogonadism**	**Mixed (primary and secondary) hypogonadism***
Congenital anorchidism Cryptorchidism Mumps orchitis Genetic and developmental conditions: Klinefelter syndrome, androgen receptor and enzyme Defects, Sertoli cell only syndrome Radiation treatment／	Genetic conditions: Kallmann's syndrome, Prader-Willi syndrome Pituitary tumours, granulomas, abscesses Hyperprolactinemia Cranial trauma Radiation treatment Various medications	Alcohol abuse Ageing Chronic infections (HIV) Corticosteroid treatment Hemochromatosis Systemic disease (liver failure, uremia, sickle-cell disease)

chemotherapy Testicular trauma Autoimmune syndromes (anti-Leydig cell disorders)		*Mixed hypogonadism is often included within the secondary hypogonadism category.

It should be noted that low testosterone can be caused by a combination of both primary and secondary hypogonadism (also called mixed hypogonadism) that reflects defects in the hypothalamus and/or the pituitary as well as the testes. This condition is frequently found in men with sickle-cell disease, thalassemia, alcoholism, glucocorticoid treatment, and in older men [7].

Due to complexity of the diagnosis of hypogonadism, there are several alternative names for male hypogonadism, e.g. androgen deficiency syndrome, androgen deficiency in the ageing male (ADAM), late-onset hypogonadism (LOH), male menopause, partial androgen decline in the ageing male or testosterone deficiency syndrome. For a purpose of this paper, the term ADAM was chosen.

Symptoms of hypogonadism

We must consider that hypogonadism is a clinical entity which is difficult to diagnose. For better understanding of the ADAM syndrome it is sometimes required to analyse also free or bioavailable testosterone [11]. However, for the initial screening of men presenting symptoms of hypogonadism, total testosterone is a reliable marker [12] (Fig. 1).

Besides the fall of testosterone levels below a physiological range, symptoms include decline in lean mass, muscle strength, adiposity, libido and erectile dysfunction, depressed mood, decreased energy or vitality, increased fatigue, osteoporosis or low bone mass, increases in body mass and overall fat mass [7, 13]. Studies of hypogonadal men shows, that there are increases in body mass and overall body fat mass as well as decreases in lean body mass with declining androgen levels [13]. Androgens also have a direct impact on bone mineral density since testosterone and oestrogens both play a vital role in bone health [14] and low testosterone levels can cause an increase in osteoclast induced bone resorption. Other sources also state direct correlation between low testosterone levels and increased risk of aortic atherosclerosis independent of age, increased body mass index (BMI), total cholesterol

or diabetes [15].

These symptoms may affect men earlier in life, already in their late third decade of the life [16]. If untreated, chronic lower than normal testosterone level dramatically increases risk of many diseases later in life. Studies have suggested a link between hypogonadism and cardiovascular disease, which is not surprising given the relationship with hypogonadism and the metabolic syndrome [9, 17]. There is a likely causal relationship between low androgen levels and aging, as well as its association with increased risk and the occurrence of cardiovascular events and progression of cardiovascular diseases [18]. On a metabolic level, men with lower androgen levels have demonstrated higher glucose and insulin levels, higher rates of obesity and increased incidence of type 2 diabetes [19 – 20], and increased risk of Alzheimer's disease [21]. There is also evidence of the importance of the androgenic hormones related to cognitive functions especially in older males [22-23] showing relationship between low testosterone levels and decrease in memory and visuospatial perception.

Testosterone replacement therapy

For more than 60 years, hypogonadism has been treated by testosterone replacement therapy (TRT) life-long, as this helps to prevent some of the adverse health effects [24-26]. Restoration of testosterone levels to the normal range improves libido, sexual function, and mood, reduces fat body mass, increases lean body mass and improves bone mineral density [3].

Among the many published trials on role of testosterone in older men, some reports increased muscle strength with testosterone replacement therapy, and some do not. Only a few reports strength gains, that can be considered substantial in comparison to the benefits of resistance exercise training. In most cases, the studies reporting significant strength gains were performed in hypogonadal subjects and employed a higher dose of testosterone, for a longer duration [27]. Nair et al. [28] described in their report treatment of a group of hypogonadal men for 24 months with a transdermal testosterone at a dose of 35 mg/week and found no increase in strength. However, 35 mg/week is less than a replacement dose and resulted in only a 30% increase in the circulating testosterone concentration. Studies by Brill et al.[29], Clague et al.[30], Kenny et al.[31], and Snyder et al.[32] also report small increases in strength. Brill et al [29] treated older men for 1 month with 5 mg testosterone/day by patch

and found an improvement in stair climb time, but no increase in strength. Clague et al. [30] treated men aged 60 or more with T of 400 ng/dL or less. The subjects were injected 200 mg testosterone enanthate every two weeks by i.m. injection for 3 months and found no significant increase in strength. Kenny et al.[31] treated hypogonadal and low-normal older men with 5 mg testosterone/day by patch for 1 year and found a 38% increase in strength with testosterone, but surprisingly also a 27% increase with placebo, with no significant difference between the two groups. Snyder et al.[32] treated older hypogonadal and eugonadal men for 36 months with 6 mg testosterone/day by patch and found no increase in strength. Maintenance of the musculo-skeletal system by increased bone density will contribute to increased physical fitness, reflected by increased strength and endurance. Treatment outcome is strongly influenced by age and training [33]. Lasaite et al. [34] observed that two-year testosterone replacement therapy in young and middle-aged hypogonadal men had beneficial effect in cognitive functioning (improved attention and visual scanning ability, executive function and psychomotor speed), but not in emotional state and quality of life. Hildreth et al.[35] found that TRT improved body composition, but it had no effect on functional performance.

Testosterone substitution can improve lipid and insulin metabolism, resulting in changes of body composition, such as decreasing fat depots and growth of muscle fibers can also be observed [33]. Sompol Permpongkosol et al.[36] in their work from 2016 found that, 8-year Treatment of long-acting testosterone undecanoate did not improve all obesity parameters. A statistically significant decrease was found in waist circumference, percentage of body fat, glycated hemoglobin, cholesterol, low-density lipoprotein, and International Prostate Symptom Score ($P < 0.05$). Testosterone undecanoate did not produce differences in body mass index, high-density lipoprotein, triglyceride, or the Aging Male Symptoms score from baseline. However, a statistically significant increase was found in the level of testosterone, prostate-specific antigen, hematocrit, International Index of Erectile Function score, and vertebral and femoral bone mineral density ($P < 0.05$). No major adverse cardiovascular events or prostate cancer occurred during this study.

Risks associated with testosterone replacement therapy (TRT)

Testosterone treatment is contraindicated in subjects with prostate and breast cancer or benign prostate hyperplasia, lower urinary tract symptoms , and if risks of treatment is perceived to be high by many physicians [3]. Other risks of TRT in men include fluid

retention, mood fluctuations, gynecomastia, worsening of sleep apnea, polycythaemia, elevation of PSA and acceleration of benign or malignant prostatic disease, oedema in patients with pre-existing cardiac, renal, or hepatic disease [3,5, 37]. Bhasin et al. [38] found high incidence of adverse effects (included haematocrit greater than 54%, leg oedema with shortness of breath, urinary retention and prostate cancer) in treating older men with the very high doses of T (300 and 600 mg/week). Rhoden and Morgentaler [39] have reviewed the adverse effects and recommend the long-term monitoring of the above-mentioned parameters. Potential adverse events not related to hormones include pain at injection site and local skin irritation.

Effects of strength training

Much research has been conducted on effect of strength protocols which incorporate large muscle groups at intensities around 70–80% of 1RM (one repetition maximum), volumes from two to three sets of 10–12 repetition, and rest periods of short duration (60–90 s) [40-41]. Beneficial effects of exercise, especially resistance training have been clearly shown on the quality of life, fatigue, muscle strength, muscular endurance and functions and body composition in elderly men with prostate cancer receiving androgen-deprivation therapy, thus being in a chronically low testosterone condition [42-43]. As for exercise interventions with ADAM patients, the scientific evidence is very limited but promising. Schwarz and Willix [18] found positive outcomes on coronary risk factors such as glucose intolerance and hyperlipidaemia when TRT was combined with endurance exercise. To our knowledge, only Hildreth et al. [35] have used resistance training and found benefits of both resistance exercise with TRT as well as without TRT in hypogonadal males. After intervention, there were no significant differences between combination of resistance exercises with TRT or with placebo in improvements in muscle function or strength in the two exercise groups. However, adding TRT resulted in greater improvements in decrease of fat mass and increase of fat-free mass. In the TRT but no exercise condition, patients did not improve muscle function but decreased fat mass, increased fat-free mass, and upper body strength. Importantly, TRT plus progressive resistance training produced greater improvements in body composition than either intervention alone.

Glintborg et al. [44] found that when TRT and strength training for six months were compared, strength training but not TRT reduced sCD36 (a plasma marker associated with atherosclerosis, insulin resistance and fatty liver in a nondiabetic healthy population) levels

suggesting decreased cardiovascular risk, possibly due to a reduction in central fat mass. Combination of exercise and TRT showed significant improvements in serum testosterone levels and symptoms of hypogonadism compared to TRT alone. In addition, these improvements were well-maintained in the combination group with continuous exercise even after cessation of TRT. Consequently, it seems like exercise can augment the durability of response to TRT and it may be the solution to shorten the treatment duration with a lower risk from testosterone therapy [45].

These are some very promising results showing a great potential of exercise in hypogonadal patients. However, the above-mentioned studies did not focus on possible physiological and metabolic mechanisms responsible for the positive effects of resistance training at circulating, cellular and molecular level. Up to date, there are no studies investigating the effects of strength training on the regulation of muscle mass and neuromuscular function on a cellular level in ADAM patients.

Aims

The overall aim of the study 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, molecular parameters of training adaptation and the quality of life of patients with ADAM.

Methods and analysis

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 a control group of healthy eugonadal males (HM), that is also engaged in strength training for 12 weeks (Fig. 2).

Participants

Subjects are included from urological units at Department of Urology, University Hospital-Petrzalka, Bratislava, Slovakia and Department of Urology, Faculty of Medicine, Comenius University, Bratislava, Slovakia. The study involves three groups of male subjects (n = 36): group 1, males with hypogonadism who are undergoing testosterone replacement therapy (TRT) (n=12); group 2, newly diagnosed males with hypogonadism without testosterone replacement therapy (NON-TRT) (n=12); group 3, healthy eugonadal men (HM)

(n=12). The subjects from all groups are engaged in strength training. The volunteers are screened before start of the participation by the urologist.

The most important inclusion criteria for participation in the study from the patient population are age 40-60 years old, subjects of hypogonadism on testosterone replacement therapy; newly diagnosed patients of hypogonadism. The most important exclusion criteria include regular strength training, conditions which are medical contraindications 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 are verbally informed about the study by their responsible urologist and the study officials usually before start of the tests. TRT provided to patients is intramuscular (IM) injection of testosterone undecanoate (TU) at a dose of 1000 mg, then repeated every 12 weeks.

Training protocol design (Intervention)

Strength training protocol starts one week after all the pre-intervention testing. The intervention is performed at the Comenius University in Bratislava, Faculty of Physical Education and Sport (FSPORT CU) in Slovakia. The strength training protocol follows a modified strength exercise program by Segal et al. [46]. The participants perform strength training sessions two times per week for 12 weeks. All training sessions are supervised and guided by professional coaches with university degree in sports training to ensure safety, correct technique and progression in training load, with a maximum of three participants per coach.

Each training session include a 5-minute general dynamic warm-up followed by progressive strength training with exercises for major muscle groups. The strength exercises are 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 participants are instructed to perform a concentric action in 2 s and immediately after an eccentric action in also 2 s. The exercises performed during every session are: leg press, split squats, bench press. The exercises altered through the week are knee extension with knee flexion, seated cable rows with seated cable pull downs, dumbbell bench press with incline dumbbell bench press (training equipment provided by KOHI Leopoldov, Slovakia and Technogym, Italia). More detailed strength training protocol can be

seen in Table 2.

During the first week of the intervention, participants are familiarized with the equipment and with the exercise technique. In the first training session, 10RM and 12RM diagnostic test for all the exercises are conducted. The training during the first 3 weeks includes mostly unilateral exercises combined with bilateral exercises. After first two weeks, the training load is gradually increased, to perform the sets with the highest load with the prescribed number of repetitions. After 3 weeks, the protocol is more focused on bilateral exercises.

Table 2: Strength training protocol

Week	Number of exercises	Number of sets	Number of repetitions	Resistance	Tempo
1 – 3. Week	3+3 (UB, LB)	1+3	10-12	10-12RM	2:0:2:1
4 – 6. week	3+3 (UB, LB)	4	10-12	10-12RM	2:0:2:1
7 – 9. week	3+3 (UB, LB)	4	6-8	6-8RM	2:0:2:1
10 – 12. week	3+3 (UB, LB)	4	6-8	6-8RM	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)

Methods:

Clinical and muscle cellular outcomes are collected before the intervention (pre-intervention assessments), after the intervention (post-training assessments). All outcomes, specific variables and assessments in each testing are listed in Additional file 2.

Clinical outcomes

Body composition is measured by Dual-energy X-ray Absorptiometry using Hologic fan-beam bone densitometer Discovery QDR series. Lean mass (LM), fat mass (FM) and total body mass (BM) is measured in arms, legs and trunk separately and total body. The changes in lower and upper body LM are investigated separately because of differences in androgen sensitivity in leg muscles compared to neck, chest and shoulder muscles [47]. Body mass is also measured by Omron BF508 body composition and body fat monitor scale, the height by stadiometer and waist circumference is measured by stretch-resistant tape that provides a constant 100 g tension. The body mass index is afterwards calculated and reported.

Muscle strength, cardiorespiratory fitness and physical functioning

Muscle strength of lower extremity is measured by maximal voluntary contraction (MVC) of isometric knee extension, MVC of isometric knee flexion and rate of force development (RFD) in isometric knee extension. These are measured by Novel Portable Isometric Knee Dynamometer (ARS dynamometry, S2P ltd., Ljubljana, Slovenia). Additionally, with awareness of health issues (such as higher blood pressure) and because of a safety reasons we predict dynamic leg press 1RM (one repetition maximum) from multiple repetition maximum testing [48] and we also measure 5 times stand-to-sit test. For upper extremities, we measure isometric MVC handgrip strength by Camry Digital Hand Dynamometer, MVC on isometric bench press using FitroDyne force plates.

Cardio-respiratory fitness is measured by The Single Stage Treadmill Walking Test on Pro Treadmill (Woodway, USA). VO_2max is calculated from results of the walking test [49]. 10-m fast walk-speed and 10-m preferred walk-speed is measured by WITTY GATE (MicroGate, Italy).

To secure validity of the physical tests, all subjects undergo a session of familiarization to the actual tests 5-7 days prior to all the intervention assessments. All sessions are performed based on the same guidelines, but after the familiarization session the load of each resistance exercise is adjusted to match the expected 1RM (one repetition maximum).

Psycho-social functioning

The general health status is 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.

Serological outcomes

Fasting morning venous blood is taken from 8:00 am to 10:00 am [50-51]. The haematological and biochemical parameters are 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.

Muscle cellular outcomes

Muscle biopsies are 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 is obtained from the mid-section of the right *m. vastus lateralis*, and after the intervention the biopsy is 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).

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.

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.

Ethics and dissemination:

The study is approved by Ethics Committee of the Derer's Memorial Hospital in Bratislava, Slovakia (ref. trial number: 127/2017) and all subjects provided and signed written informed consent. Trial registration: ClinicalTrials.gov: NCT03282682

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Competing interests statement

We declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

Figures

Figure 1: An algorithm for the diagnosis of hypogonadism (T - Total Testosterone) according to 7, 52-53. (Adapted from [3]).

Figure 2: Timeline of the ADAM study.

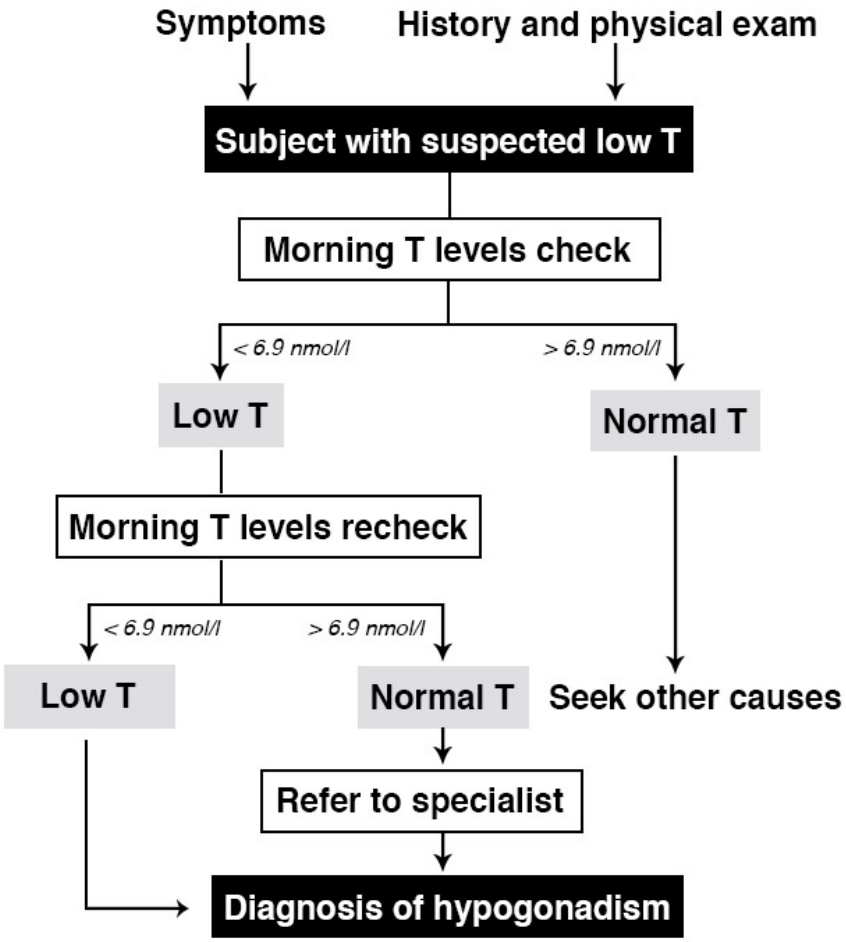

Figure 1: An algorithm for the diagnosis of hypogonadism (T - Total Testosterone) according to 7, 52-53. (Adapted from [3]).

126x137mm (144 x 144 DPI)

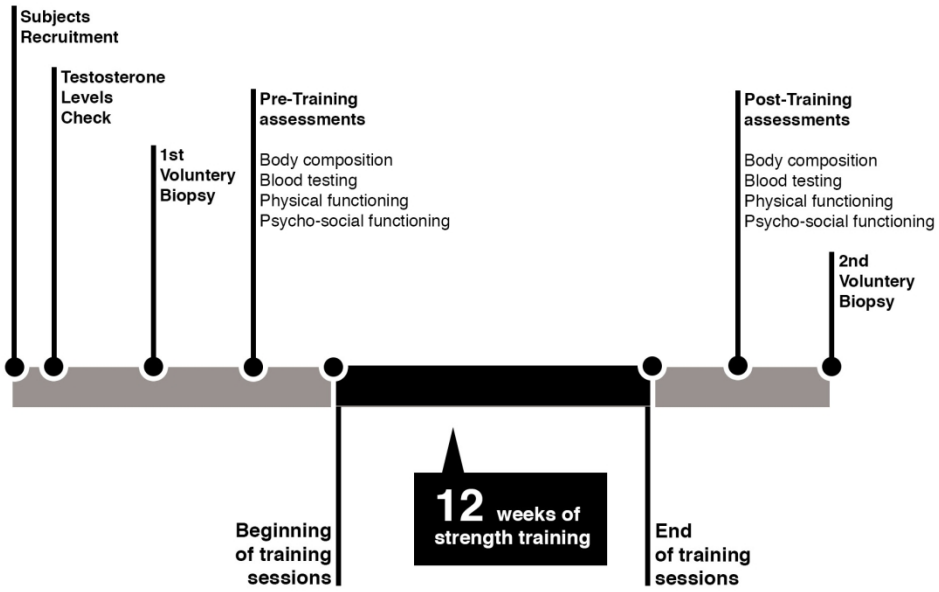

Figure 2: Timeline of the ADAM study.

250x184mm (200 x 200 DPI)

Additional file 1: Inclusion and exclusion criteria

Subject's Code: | | | | | | Subject's Date of Birth:

Subject's Name:

Inclusion criteria

1A. Newly diagnosed with ADAM syndrome ☐ Yes ☐ No
1B. Patient with ADAM syndrome on testosterone replacement therapy ☐ Yes ☐ No
2. 40 - 60 years of age ☐ Yes ☐ No
3. Capable of reading and writing Slovak ☐ Yes ☐ No
4. Treating urologist\endocrinologist has approved the subjects'participation ☐ Yes ☐ No
4. Lives within approximately 1 hour from Bratislava by car of public transportation ☐ Yes ☐ No
6. Written informed consent received ☐ Yes ☐ No

Exclusion criteria

1. Rutine resistance training with manuals ☐ Yes ☐ No
2. Medication for osteoporosis ☐ Yes ☐ No
3. Conditions that contraindicate exercise without adjusted actions ☐ Yes ☐ No
4. Mentally incompetent conditions ☐ Yes ☐ No
5. Conditions complicating ability to participate in a supervised training program ☐ Yes ☐ No
6. Abnormal DRV (digital rectal examination) ☐ Yes ☐ No
7. Serious system deases as
   a) cardiovascular deases ☐ Yes ☐ No
   b) liver and kidneys deases, ☐ Yes ☐ No
   c) diabetes mellius, ☐ Yes ☐ No
   d) oncological deases ☐ Yes ☐ No
   e) or other serious dease according to the judgment of the responsible physician. ☐ Yes ☐ No

Clinician's Signature: _____ Date:

Additional file 2: Outcomes, specific variables and assessments

Outcomes	Specific variables	Assessments
CLINICAL OUTCOMES		
Body composition		
	Lean Mass (LM) – primary outcome	DXA
	Fat Mass (FM)	DXA
	Total Body Mass (TBM)	DXA
	Body Mass Index (BMI)	Weight and height
The haematological and biochemical parameters	Haemoglobin(g/l), Hematocrits(ratio), Leucocytes (10^9/l), Thrombocytes (10^9/l), Glucose (mmol/l), Urea (mmol/l), Sodium (mmol/l), Potassium (mmol/l), Calcium (mmol/l), ALAT, Total Cholesterol (mmol/l), LDL Cholesterol (mmol/l), HDL Cholesterol (mmol/l), Triglyceride (mmol/l), Testosterone (nmol/l), Oestrogen (nmol/l), LH (nmol/l), FSH (nmol/l), SHBG (nmol/l), Albumin (g/l), Bilirubin (µmol/l), Total Protein (g/l), CRP(mg/l), Insulin (mIU/l), PSA (ug/l).	
Physical functioning		
	Muscle strength	10-m Usual Walk Test (s) 10-m Fast Walk Test (s) Maximal voluntary contraction (MVC) of isometric knee extension (Nm) Maximal voluntary contraction (MVC) of isometric knee flexion (Nm) Change in maximal voluntary contraction (MVC) in bench press (kg) 1RM on leg press (kg) Isometric 1RM Bench-press test (kg) Handgrip strength (kg)
	Cardio-respiratory fitness	The Single Stage Treadmill Walking Test (VO2max in ml.kg-1.min-1)
Psycho-social functioning		
	Symptoms of ADAM	Aging Males' Symptom (AMS) Scale
	HRQoL	SF-36
Muscle cellular outcomes		
Muscle fibre size	Muscle fibre cross sectional area (primary cellular outcome)	Cross sections of muscle biopsies
Regulators of muscle fibre size		
Number of myonuclei per muscle fibre		Number of myonuclei per muscle fibre

Number of satellite cells per muscle fibre		Cross sections of muscle biopsies
Number of satellite cells and myonuclei positive for androgen receptors		Cross sections of muscle biopsies

DXA - Dual-energy X-ray Absorptiometry, LDL - Low Density Lipoprotein, HDL - High Density Lipoprotein, LH - Luteinizing Hormone, FSH - Follicle Stimulating Hormone, SHBG - Sex Hormone-Binding Globulin, CRP - C- Reactive Protein, PSA - Prostate Specific Antigen, ADAM – androgen deficiency in aging male, HRQoL – Health-Related Quality of Life.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

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

BMJ Open

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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Secondary Subject Heading:	Urology, Sports and exercise medicine, Research methods
Keywords:	hypogonadism, testosterone deficiency, strength training, testosterone, physical activity, aging male

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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Abstract

Introduction: Androgen deficiency of the aging male (ADAM) is a clinical syndrome resulting from the low production of androgens (testosterone levels <6.9 nmol/l) with symptoms including decline in lean mass, muscle strength, increases in body mass and overall fat mass. The aim of the study is to examine the effect of a 12-week strength training intervention on body composition, physical function, muscle cellular and molecular and selected biochemical markers of metabolic health in hypogonadal patients.

Methods and analysis: The study is 3-group controlled 12-week experiment to assess the effect of strength training (ST) on hypogonadal patients with testosterone replacement therapy and newly diagnosed males without TRT. Age matched healthy eugonadal males are also engaged in strength training. All outcomes are collected before the intervention (pre-intervention assessments) and after the intervention (post-intervention assessments). Clinical outcomes are body composition (lean mass, fat mass, and total body mass) measured by Dual-energy X-ray Absorptiometry (DXA), physical functioning (muscle strength, cardio-respiratory fitness) assessed by physical tests and psycho-social functioning. The most important haematological and biochemical parameters included are glucose, total cholesterol, LDL cholesterol, HDL cholesterol, testosterone, LH, FSH, SHBG, total protein, CRP, insulin and PSA. Muscle cellular and molecular outcomes are muscle fiber size, regulators of muscle fibber size and regulators of muscle fiber function. Muscle cellular outcomes are measured on muscle cross sections and muscle homogenate from muscle biopsies obtained from m. vastus lateralis.

Ethics and Dissemination: This trial is approved by Ethics Committee of the University Hospital in Bratislava, Slovakia (ref. trial number: 127/2017) and all subjects will be fully informed on the rationale, risks and benefits of the study and sign the written informed consent prior entering the study. Results will be published in peer-reviewed journals, presented in scientific conferences and disseminated to healthcare professional. Trial registration: ClinicalTrials.gov: NCT03282682.

Strengths and limitations of this study

- To the best of our knowledge this trial represents the first study in hypogonadal males focusing on possible physiological and metabolic mechanisms of strength training at circulating, cellular and molecular level.

- Wide spectrum of clinical outcomes with high-standard methods of assessments (DXA, muscle biopsies).

- The major limitation of this trial is small sample size, caused by limited number of detected patients.

Introduction

Testosterone is one of the most potent naturally secreted androgenic-anabolic hormone, and its biological effects include, among others, promotion of skeletal muscle growth [1]. Testosterone stimulates protein synthesis, inhibits protein degradation and these effects account for the promotion of muscle hypertrophy by testosterone [2]. Aging beyond 35–40 years is associated with a decline of 1–3% per year in circulating testosterone concentration (1.6% in total and 2–3% in bioavailable testosterone) in men. This reduction can eventually lead to very low resting concentrations of circulating testosterone, a condition that has been termed andropause [3-6].

Although the lower limit of normal total testosterone is not clearly defined, American Association of Clinical Endocrinologists (AACE) suggests 6.9 nmol/l as lower limit of normal testosterone levels, other societies suggest 8 nmol/l and even up to 10 nmol/l as a limit below which patients can be considered as hypogonadal. The Endocrine Society defines male hypogonadism as a clinical syndrome resulting from failure of the testis to produce physiological levels of testosterone (androgen deficiency) and normal number of spermatozoa. Hypogonadism (primary, secondary or mixed) is caused by disruption of one or more levels of the hypothalamic–pituitary–gonadal axis [7]. All the causes of male hypogonadism can be found in Table 1[8-10]. Due to complexity of the diagnosis of hypogonadism, there are several alternative names for male hypogonadism, but for a purpose of this trial, the term ADAM was chosen.

Table 1: Causes of male hypogonadism

Primary hypogonadism	Secondary hypogonadism	Mixed (primary and secondary) hypogonadism*
Congenital anorchidism Cryptorchidism Mumps orchitis	Genetic conditions: Kallmann's syndrome, Prader-Willi syndrome	Alcohol abuse Ageing Chronic infections (HIV)

Genetic and developmental conditions: Klinefelter syndrome, androgen receptor and enzyme Defects, Sertoli cell only syndrome Radiation treatment/chemotherapy Testicular trauma Autoimmune syndromes (anti-Leydig cell disorders)	Pituitary tumours, granulomas, abscesses Hyperprolactinemia Cranial trauma Radiation treatment Various medications	Corticosteroid treatment Hemochromatosis Systemic disease (liver failure, uremia, sickle-cell disease) *Mixed hypogonadism is often included within the secondary hypogonadism category.

Symptoms of hypogonadism

Total testosterone is a reliable marker for the initial screening of men presenting symptoms of hypogonadism, [11-13] (Fig. 1), but for better understanding of the ADAM syndrome it is sometimes required to analyse also free or bioavailable testosterone [14].

Symptoms of male hypogonadism include decline in lean mass (LM), muscle strength, adiposity, libido and erectile dysfunction, depressed mood, decreased energy or vitality, increased fatigue, osteoporosis or low bone mass, increases in body mass and overall fat mass [7, 15]. Studies of hypogonadal men shows, that there are increases in body mass and overall body fat mass as well as decreases in LM with declining androgen levels [15]. Androgens also have a direct impact on bone mineral density since testosterone and oestrogens both play a vital role in bone health and low testosterone levels can cause an increase in osteoclast induced bone resorption [16]. Other sources also state direct correlation between low testosterone levels and increased risk of aortic atherosclerosis independent of age, increased body mass index (BMI), total cholesterol or diabetes [17].

These symptoms may affect men earlier in life, already in their late third decade of life [18]. If untreated, chronic lower than normal testosterone level dramatically increases risk of many diseases later in life. Studies have suggested a link between hypogonadism and cardiovascular disease, which is not surprising given the relationship with hypogonadism and the metabolic syndrome [9, 19]. There is a likely causal relationship between low androgen levels and aging, as well as its association with increased risk and the occurrence of cardiovascular events and progression of cardiovascular diseases [20]. On a metabolic level, men with lower androgen levels have demonstrated higher glucose and insulin levels, higher rates of obesity and increased incidence of type 2 diabetes and other diseases [21 – 25].

Testosterone replacement therapy

For decades hypogonadism has been treated by testosterone replacement therapy (TRT) life-long, as this helps to prevent some of the adverse health effects [26-28]. Restoration of testosterone levels to the normal range improves libido, sexual function, and mood, reduces fat body mass, increases lean body mass and improves bone mineral density [3].

Among the published trials on the role of testosterone in older men, not all report increased muscle strength with testosterone replacement therapy. The studies reporting significant strength gains were performed in hypogonadal subjects and employed a higher dose of testosterone for a longer duration [29]. Nair et al. [30] described in their report treatment of a group of hypogonadal men with a transdermal testosterone at a dose of 35 mg/week for 24 months and found no increase in strength. However, 35 mg/week is less than a replacement dose and resulted in only a 30% increase in the circulating testosterone concentration. Some other studies [31-34] also report small or no increases in muscle strength with TRT. Maintenance of the musculo-skeletal system by increased bone density will contribute to increased physical fitness, reflected by increased strength and endurance [35], and the treatment outcome is strongly influenced by age and training [35]. Lasaite et al. [36] observed that two-year testosterone replacement therapy in young and middle-aged hypogonadal men had beneficial effect on cognitive functioning (improved attention and visual scanning ability, executive function and psychomotor speed), but not on emotional state and quality of life. Hildreth et al. [37] found that TRT improved body composition, but it had no effect on functional performance. Testosterone replacement can improve lipid and insulin metabolism, resulting in changes of body composition, such as decreasing fat depots and growth of muscle fibers can also be observed [36]. Permpongkosol et al. [38] in their work from 2016 found that 8-year treatment of long-acting testosterone undecanoate did not improve all obesity parameters.

It is still not clear how testosterone effects cognitive function in adult men, but testosterone may exert its action through androgen receptors in the brain and has been shown effect on serotonin, dopamine, acetylcholine, and calcium signaling [39]. Barrett-Connor et al. [40] found correlation between higher bioavailable testosterone and better scores on 2 of 12 cognitive function tests. Higher total or bioavailable testosterone levels tended to be associated with better performance on tests with verbal memory and mental control. Testosterone enhanced cerebral perfusion in hypogonadal men and that perfusion takes place specifically in Brodman areas 8 and 24, regions of the brain that are concerned with: strategic planning, higher motor action, cognitive behaviors, emotional behavior, generalized emotional reaction,

wakefulness and memory [41]. Hypogonadal men have lower scores in tests of memory, visuospatial function, with a faster decline in visual memory [42]. McIntyre et al. [43] found, that middle-aged males with depressions did have a reduction in bio-available testosterone.

Risks associated with testosterone replacement therapy (TRT)

Testosterone treatment is contraindicated in subjects with breast cancer or benign prostate hyperplasia, lower urinary tract symptoms, and if risks of treatment is perceived to be high by many physicians [3]. The risk of prostate cancer with TRT is still unclear. Only intra-muscular treatment found slight increase in PSA levels [44]. Loeb et al. [45] found that TRT remained significantly associated with more favourable-risk prostate cancer and lower risk of aggressive prostate cancer. But other studies and meta-analysis found TRT as a safe urological approach to treat hypogonadism [46-47]. Other risks of TRT in men include fluid retention, mood fluctuations, gynecomastia, worsening of sleep apnea, polycythaemia, elevation of PSA [3,5, 48]. Bhasin et al. [49] found higher incidence of adverse effects (included haematocrit greater than 54%, leg oedema with shortness of breath, urinary retention and prostate cancer) in treating older men with the very high doses of T compared to young males. Rhoden and Morgentaler [50] have reviewed the adverse effects and recommend the long-term monitoring of the above-mentioned parameters. Potential adverse events not related to hormones include pain at injection site and local skin irritation.

The effects of strength training

Much research has been conducted on the effect of strength protocols which incorporate large muscle groups at intensities around 70–80% of 1RM (one repetition maximum), volumes from two to three sets of 10–12 repetition, and rest periods of short to medium duration (60–90 s) [51-52]. Beneficial effects of exercise, especially resistance training have been clearly shown with regards to the quality of life, fatigue, muscle strength, muscular endurance and functions and body composition in elderly men with prostate cancer receiving androgen-deprivation therapy, thus being in a chronically low testosterone condition [53-54]. Clearly, resting levels of testosterone and other androgens but not their acute elevations due to exercise have also impact on muscle hypertrophy as suggested by a recent review article [55]. As for exercise interventions with ADAM patients, the scientific evidence is very limited but promising. Schwarz and Willix [20] found positive outcomes on coronary risk factors such as glucose intolerance and hyperlipidaemia when TRT was combined with endurance exercise. To our

knowledge, only Hildreth et al. [37] have used resistance training and found benefits of both resistance exercise with TRT as well as without TRT in hypogonadal males. After intervention, there were no significant differences between combination of resistance exercises with TRT or with placebo in improvements in muscle function or strength in the two exercise groups. However, adding TRT resulted in greater improvements in decrease of fat mass and increase of fat-free mass. In the TRT but no exercise condition, patients did not improve muscle function but decreased fat mass, increased fat-free mass, and upper body strength. Importantly, TRT plus progressive resistance training produced greater improvements in body composition than either intervention alone.

Glintborg et al. [56] studied effects of TRT and/or strength training (ST) on cardiovascular risk in hypogonadal males for 6 months. This double-blinded, placebo-controlled study found that only ST + placebo significantly decreased sCD36 levels. Only placebo group did not decrease fat mass during this period. Compared to TRT, six months of strength training reduced sCD36 levels suggesting decreased cardiovascular risk, possibly due to a reduction in central fat mass.

In a pilot randomized controlled trial by Cho and colleagues [57] when hypogonadal males were treated with combination of exercise and TRT, significantly better results in serum testosterone levels and symptoms of hypogonadism compared to TRT alone after 12 weeks of intervention were found. The levels of testosterone were significantly higher in the combination group ($p = 0.01$) In addition, these improvements were well-maintained in the combination group with continuous exercise even after cessation of TRT. After 20 weeks of intervention the group which used TRT and strength training kept the testosterone levels significantly higher ($p = 0.01$) compared to the group with TRT only. Consequently, it seems that exercise can augment the durability of response to TRT and it may be the solution to shorten the treatment duration with a lower risk from testosterone therapy [57]. There are some very promising results showing a great potential of exercise in hypogonadal patients. However, the above-mentioned studies did not focus on possible physiological and metabolic mechanisms responsible for the positive effects of resistance training at circulating, cellular and molecular level. Up to date, there are no studies investigating the effects of strength training on the regulation of muscle mass and neuromuscular function at a cellular level in hypogonadal male patients.

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 (Fig. 2).

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. [58] 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 general 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: General dynamic warm-up

General dynamic Warmup 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. squats) develop similar magnitude of muscle activity with producing less load on the spine, thus they are safer [59], 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. At the end of the session, the trainers will ask participants if they experienced any adverse events during the session, which will be also recorded. 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 [60]. Due to very similar results but greater participant comfort [61] 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. [61] 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 [62].

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 [63], 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 [63].

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 [64]. 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. [65].

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 [66] 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. [67] (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 will be performed using a statistical software and *P* values < 0.05 will be considered significant. Data will be presented as means and standard deviations.

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 the a research team member.

Patients or public (patient organisations) 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

*T*he 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, DEXA) and that of maximal voluntary contraction force measured on linear leg-press (31% increase, p<0.0001, 1RM) - were 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 changes lean body mass at the power of 0.90.

Ethics and Dissemination

This trial is funded by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and of the Slovak Academy of Sciences (VEGA) no. 1/0714/16. 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

We declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

Figures

Figure 1: An algorithm for the diagnosis of hypogonadism (T - Total Testosterone) according to 7, 12-13. (Adapted from [3]).

Figure 2: Timeline of the ADAM study.

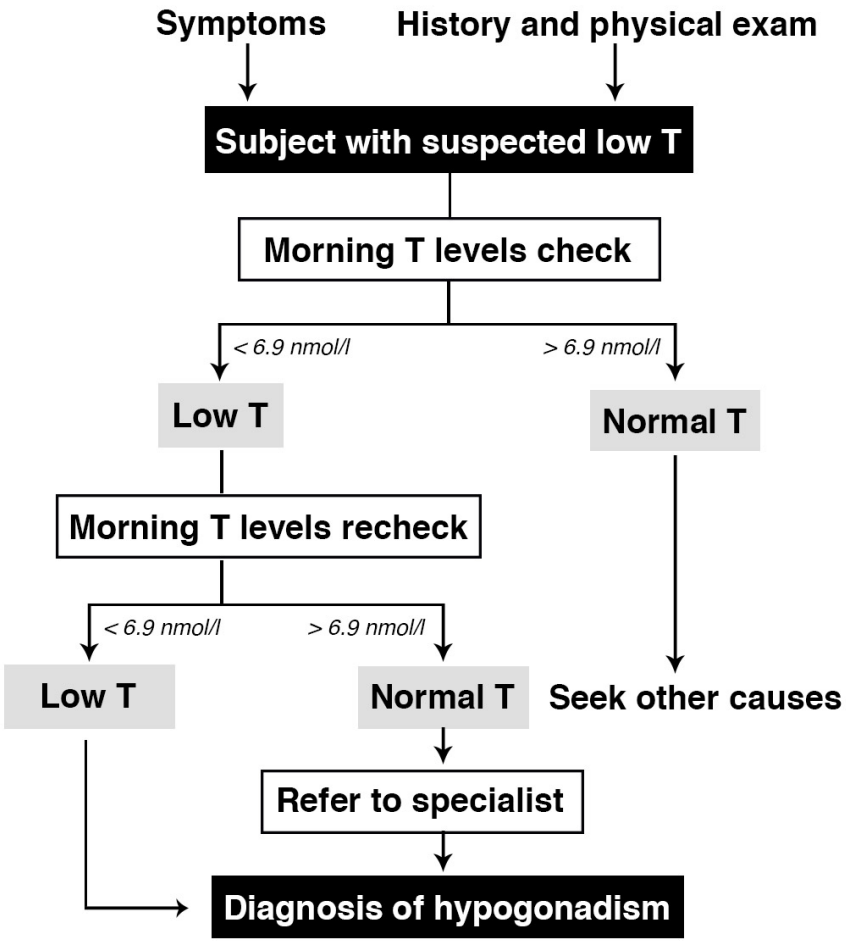

Figure 1: An algorithm for the diagnosis of hypogonadism (T - Total Testosterone) according to 7, 12-13. (Adapted from [3]).

99x108mm (300 x 300 DPI)

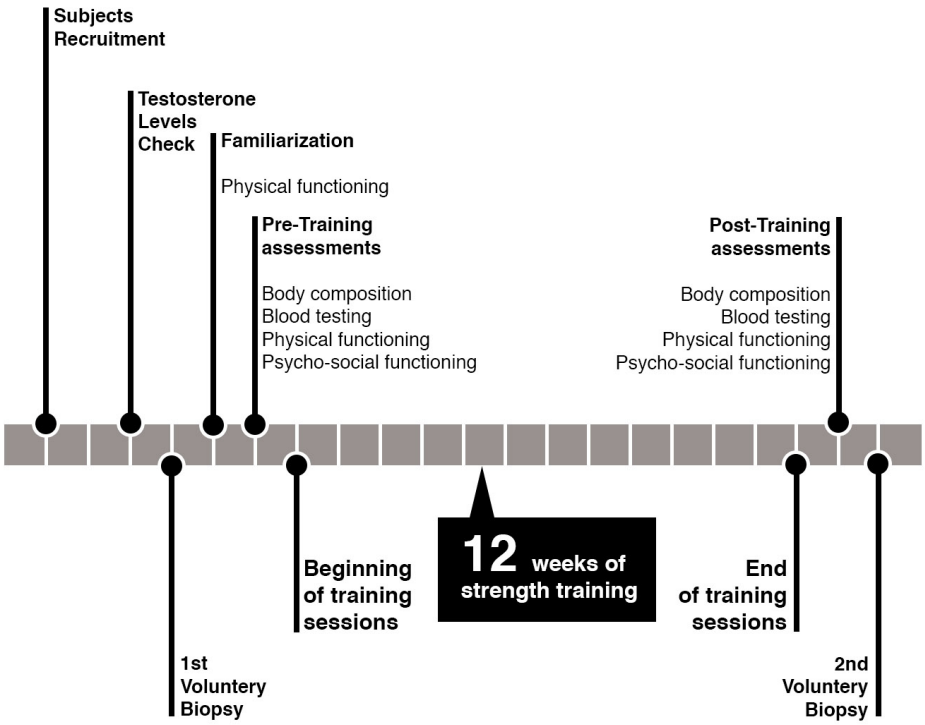

Figure 2: Timeline of the ADAM study.

119x99mm (300 x 300 DPI)

Additional file 1: Inclusion and exclusion criteria

Subject's Code: | | | | | | Subject's Date of Birth:

Subject's Name:

Inclusion criteria

1A. Newly diagnosed with ADAM syndrome ☐ Yes ☐ No
1B. Patient with ADAM syndrome on testosterone replacement therapy ☐ Yes ☐ No
2. 40 - 60 years of age ☐ Yes ☐ No
3. Capable of reading and writing Slovak ☐ Yes ☐ No
4. Treating urologist\endocrinologist has approved the subjects'participation ☐ Yes ☐ No
4. Lives within approximately 1 hour from Bratislava by car of public transportation ☐ Yes ☐ No
6. Written informed consent received ☐ Yes ☐ No

Exclusion criteria

1. Rutine resistance training with manuals ☐ Yes ☐ No
2. Medication for osteoporosis ☐ Yes ☐ No
3. Conditions that contraindicate exercise without adjusted actions ☐ Yes ☐ No
4. Mentally incompetent conditions ☐ Yes ☐ No
5. Conditions complicating ability to participate in a supervised training program ☐ Yes ☐ No
6. Abnormal DRV (digital rectal examination) ☐ Yes ☐ No
7. Serious system deases as
   a) cardiovascular deases ☐ Yes ☐ No
   b) liver and kidneys deases, ☐ Yes ☐ No
   c) diabetes mellius, ☐ Yes ☐ No
   d) oncological deases ☐ Yes ☐ No
   e) or other serious dease according to the judgment of the responsible physician. ☐ Yes ☐ No

Clinician's Signature: _____ Date:

Additional file 2: Outcomes, specific variables and assessments

Outcomes	Specific variables	Assessments
CLINICAL OUTCOMES		
Body composition		
	Lean Mass (LM) – primary outcome	DXA
	Fat Mass (FM)	DXA
	Total Body Mass (TBM)	DXA
	Body Mass Index (BMI)	Weight and height
The haematological and biochemical parameters	Haemoglobin(g/l), Hematocrits(ratio), Leucocytes (10^9/l), Thrombocytes (10^9/l), Glucose (mmol/l), Urea (mmol/l), Sodium (mmol/l), Potassium (mmol/l), Calcium (mmol/l), ALAT, Total Cholesterol (mmol/l), LDL Cholesterol (mmol/l), HDL Cholesterol (mmol/l), Triglyceride (mmol/l), Testosterone (nmol/l), Oestrogen (nmol/l), LH (nmol/l), FSH (nmol/l), SHBG (nmol/l), Albumin (g/l), Bilirubin (µmol/l), Total Protein (g/l), CRP(mg/l), Insulin (mIU/l), PSA (ug/l).	
Physical functioning		
	Muscle strength	10-m Usual Walk Test (s) 10-m Fast Walk Test (s) Maximal voluntary contraction (MVC) of isometric knee extension (Nm) Maximal voluntary contraction (MVC) of isometric knee flexion (Nm) 1RM on leg press (kg) Handgrip strength (kg)
	Cardio-respiratory fitness	The Single Stage Treadmill Walking Test (VO2max in ml.kg-1.min-1)
Psycho-social functioning		
	Symptoms of ADAM	Aging Males' Symptom (AMS) Scale
	HRQoL	SF-36
Muscle cellular outcomes		
Muscle fibre size	Muscle fibre cross sectional area (primary cellular outcome)	Cross sections of muscle biopsies
Regulators of muscle fibre size		
Number of myonuclei per muscle fibre		Number of myonuclei per muscle fibre
Number of satellite cells per muscle fibre		Cross sections of muscle biopsies

Number of satellite cells and myonuclei positive for androgen receptors		Cross sections of muscle biopsies

DXA - Dual-energy X-ray Absorptiometry, LDL - Low Density Lipoprotein, HDL - High Density Lipoprotein, LH - Luteinizing Hormone, FSH - Follicle Stimulating Hormone, SHBG - Sex Hormone-Binding Globulin, CRP - C- Reactive Protein, PSA - Prostate Specific Antigen, ADAM – androgen deficiency in aging male, HRQoL – Health-Related Quality of Life.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

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

BMJ Open

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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Secondary Subject Heading:	Urology, Sports and exercise medicine, Research methods
Keywords:	hypogonadism, testosterone deficiency, strength training, testosterone, physical activity, aging male

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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Abstract

Introduction: Androgen deficiency of the aging male is a clinical syndrome resulting from the low production of androgens (testosterone levels <6.9 nmol/l) with symptoms including decline in lean mass, muscle strength, increases in body mass and overall fat mass. The aim of the study is to examine the effect of a 12-week strength training intervention on body composition, physical function, muscle cellular and molecular and selected biochemical markers of metabolic health in hypogonadal patients.

Methods and analysis: The study is 3-group controlled 12-week experiment to assess the effect of strength training (ST) on hypogonadal patients with testosterone replacement therapy and newly diagnosed males without TRT. Age matched healthy eugonadal males are also engaged in strength training. Lean mass is used to determine sample size indicating, that 22 subjects per group will be sufficient to detect intervention related changes at the power of 0.90. All outcomes are collected before the intervention (pre-intervention assessments) and after the intervention (post-intervention assessments). Clinical outcomes are body composition (lean mass, fat mass, and total body mass) measured by Dual-energy X-ray Absorptiometry, physical functioning assessed by physical tests and psycho-social functioning. The most important haematological and biochemical parameters included are glucose, total cholesterol, LDL cholesterol, HDL cholesterol, testosterone, LH, FSH, SHBG, insulin and PSA. Muscle cellular and molecular outcomes are muscle fiber size and regulators of muscle fibber size. Muscle cellular outcomes are measured from muscle biopsies obtained from m. vastus lateralis.

Ethics and Dissemination: This trial is approved by Ethics Committee of the University Hospital in Bratislava, Slovakia (ref. trial number: 127/2017) and all subjects will be fully informed on the rationale, risks and benefits of the study and sign the written informed consent prior entering the study. Results will be published in peer-reviewed journals and presented in scientific conferences. Trial registration: ClinicalTrials.gov: NCT03282682.

Strengths and limitations of this study

- To the best of our knowledge this trial represents the first study in hypogonadal males focusing on possible physiological and metabolic mechanisms of strength training at circulating, cellular and molecular level.

- Wide spectrum of clinical outcomes with high-standard methods of assessments (DXA, muscle biopsies).

- The major limitation of this trial is small sample size, caused by limited number of detected patients.
- Another limitation is that the participants will be asked to not change their habitual dietary intake during the intervention, but the actual intake is not monitored.

Introduction

Testosterone is one of the most potent naturally secreted androgenic-anabolic hormone, and its biological effects include, among others, promotion of skeletal muscle growth [1]. Testosterone stimulates protein synthesis, inhibits protein degradation and these effects account for the promotion of muscle hypertrophy by testosterone [2]. Aging beyond 35–40 years is associated with a decline of 1–3% per year in circulating testosterone concentration (1.6% in total and 2–3% in bioavailable testosterone) in men. This reduction can eventually lead to very low resting concentrations of circulating testosterone, a condition that has been termed andropause [3-6].

Although the lower limit of normal total testosterone is not clearly defined, American Association of Clinical Endocrinologists (AACE) suggests 6.9 nmol/l as lower limit of normal testosterone levels, other societies suggest 8 nmol/l and even up to 10 nmol/l [7] as a limit below which patients can be considered as hypogonadal. The Endocrine Society defines male hypogonadism as a clinical syndrome resulting from failure of the testis to produce physiological levels of testosterone (androgen deficiency) and normal number of spermatozoa. Hypogonadism (primary, secondary or mixed) is caused by disruption of one or more levels of the hypothalamic–pituitary–gonadal axis [8]. All the causes of male hypogonadism can be found in Table 1[9-11]. Due to complexity of the diagnosis of hypogonadism, there are several alternative names for male hypogonadism, but for a purpose of this trial, the term ADAM was chosen.

Table 1: Causes of male hypogonadism

Primary hypogonadism	Secondary hypogonadism	Mixed (primary and secondary) hypogonadism*
Congenital anorchidism Cryptorchidism Mumps orchitis Genetic and developmental conditions: Klinefelter syndrome, androgen receptor	Genetic conditions: Kallmann's syndrome, Prader-Willi syndrome Pituitary tumours, granulomas, abscesses Hyperprolactinemia	Alcohol abuse Ageing Chronic infections (HIV) Corticosteroid treatment

and enzyme Defects, Sertoli cell only syndrome Radiation treatment/chemotherapy Testicular trauma Autoimmune syndromes (anti-Leydig cell disorders)	Cranial trauma Radiation treatment Various medications	Hemochromatosis Systemic disease (liver failure, uremia, sickle-cell disease) *Mixed hypogonadism is often included within the secondary hypogonadism category.

Symptoms of hypogonadism

Total testosterone is a reliable marker for the initial screening of men presenting symptoms of hypogonadism, [12-14] (Fig. 1), but for better understanding of the ADAM syndrome it is sometimes required to analyse also free or bioavailable testosterone [15]. Most testosterone circulates tightly bound to sex hormone-binding globulin (SHBG) or weakly bound to albumin. A minor amount circulates as free testosterone, and it is believed that this is the metabolically active fraction. Therefore, measurements of free testosterone is important in the diagnosis of disorders of androgen deficiency in men [16].

Symptoms of male hypogonadism include decline in lean mass (LM), muscle strength, adiposity, libido and erectile dysfunction, depressed mood, decreased energy or vitality, increased fatigue, osteoporosis or low bone mass, increases in body mass and overall fat mass [8, 17]. Studies of hypogonadal men shows, that there are increases in body mass and overall body fat mass as well as decreases in LM with declining androgen levels [17]. Androgens also have a direct impact on bone mineral density since testosterone and oestrogens both play a vital role in bone health and low testosterone levels can cause an increase in osteoclast induced bone resorption [18]. Other sources also state direct correlation between low testosterone levels and increased risk of aortic atherosclerosis independent of age, increased body mass index (BMI), total cholesterol or diabetes [19].

These symptoms may affect men earlier in life, already in their late third decade of life [20]. If untreated, chronic lower than normal testosterone level dramatically increases risk of many diseases later in life. Studies have suggested a link between hypogonadism and cardiovascular disease, which is not surprising given the relationship with hypogonadism and the metabolic syndrome [10, 21]. Testosterone is a hormone regulating several pathways affecting many other syndromes, for example locomotive syndrome [22]. There is a likely causal relationship between low androgen levels and aging, as well as its association with increased risk and the

occurrence of cardiovascular events and progression of cardiovascular diseases [23]. On a metabolic level, men with lower androgen levels have demonstrated higher glucose and insulin levels, higher rates of obesity and increased incidence of type 2 diabetes and other diseases [24 – 28].

Testosterone replacement therapy

For decades hypogonadism, has been treated by testosterone replacement therapy (TRT) life-long, as this helps to prevent some of the adverse health effects [29-31]. Restoration of testosterone levels to the normal range improves libido, sexual function, and mood, reduces fat body mass, increases lean body mass and improves bone mineral density [3].

Among the published trials on the role of testosterone in older men, not all report increased muscle strength with testosterone replacement therapy. The studies reporting significant strength gains were performed in hypogonadal subjects and employed a higher dose of testosterone for a longer duration [32]. Nair et al. [33] described in their report treatment of a group of hypogonadal men with a transdermal testosterone at a dose of 35 mg/week for 24 months and found no increase in strength. However, 35 mg/week is less than a replacement dose and resulted in only a 30% increase in the circulating testosterone concentration. Some other studies [34-37] also report small or no increases in muscle strength with TRT. Maintenance of the musculoskeletal system by increased bone density will contribute to increased physical fitness, reflected by increased strength and endurance [38], and the treatment outcome is strongly influenced by age and training [38]. Lasaite et al. [39] observed that two-year testosterone replacement therapy in young and middle-aged hypogonadal men had beneficial effect on cognitive functioning (improved attention and visual scanning ability, executive function and psychomotor speed), but not on emotional state and quality of life. Hildreth et al. [40] found that TRT improved body composition, but it had no effect on functional performance. Testosterone replacement can improve lipid and insulin metabolism, resulting in changes of body composition, such as decreasing fat depots and growth of muscle fibers can also be observed [39]. Permpongkosol et al. [41] in their work from 2016 found that 8-year treatment of long-acting testosterone undecanoate did not improve all obesity parameters.

It is still not clear how testosterone effects cognitive function in adult men, but testosterone may exert its action through androgen receptors in the brain and has been shown effect on

serotonin, dopamine, acetylcholine, and calcium signalling [42]. Barrett-Connor et al. [43] found correlation between higher bioavailable testosterone and better scores on 2 of 12 cognitive function tests. Higher total or bioavailable testosterone levels tended to be associated with better performance on tests with verbal memory and mental control. Testosterone enhanced cerebral perfusion in hypogonadal men and that perfusion takes place specifically in Brodman areas 8 and 24, regions of the brain that are concerned with: strategic planning, higher motor action, cognitive behaviours, emotional behaviour, generalized emotional reaction, wakefulness and memory [44]. Hypogonadal men have lower scores in tests of memory, visuospatial function, with a faster decline in visual memory [45]. McIntyre et al. [46] found, that middle-aged males with depressions did have a reduction in bio-available testosterone.

Risks associated with testosterone replacement therapy (TRT)

Testosterone treatment is contraindicated in subjects with breast cancer or benign prostate hyperplasia, lower urinary tract symptoms, and if risks of treatment is perceived to be high by many physicians [3]. The risk of prostate cancer with TRT is still unclear. Only intra-muscular treatment found slight increase in PSA levels [47]. Loeb et al. [48] found that TRT remained significantly associated with more favourable-risk prostate cancer and lower risk of aggressive prostate cancer. But other studies and meta-analysis found TRT as a safe urological approach to treat hypogonadism [49-51]. Other risks of TRT in men include fluid retention, mood fluctuations, gynecomastia, worsening of sleep apnea, polycythaemia, elevation of PSA [3,5, 51]. Bhasin et al. [52] found higher incidence of adverse effects (included haematocrit greater than 54%, leg oedema with shortness of breath, urinary retention and prostate cancer) in treating older men with the very high doses of T compared to young males. Rhoden and Morgentaler [53] have reviewed the adverse effects and recommend the long-term monitoring of the above-mentioned parameters. Potential adverse events not related to hormones include pain at injection site and local skin irritation.

The effects of strength training

Much research has been conducted on the effect of strength protocols on muscle mass and muscle strength which incorporate large muscle groups at intensities around 70–80% of 1RM (one repetition maximum), volumes from two to three sets of 10–12 repetition, and rest periods of short to medium duration (60–90 s) [54-55]. Beneficial effects of exercise, especially resistance training have been clearly shown with regards to the quality of life, fatigue, muscle

strength, muscular endurance and functions and body composition in elderly men with prostate cancer receiving androgen-deprivation therapy, thus being in a chronically low testosterone condition [56-57]. Clearly, resting levels of testosterone and other androgens but not their acute elevations due to exercise have also impact on muscle hypertrophy as suggested by a recent review article [58]. As for exercise interventions with ADAM patients, the scientific evidence is very limited but promising. Schwarz and Willix [23] found positive outcomes on coronary risk factors such as glucose intolerance and hyperlipidaemia when TRT was combined with endurance exercise. To our knowledge, only Hildreth et al. [40] have used resistance training and found benefits of both resistance exercise with TRT as well as without TRT in hypogonadal males. After intervention, there were no significant differences between combination of resistance exercises with TRT or with placebo in improvements in muscle function or strength in the two exercise groups. However, adding TRT resulted in greater improvements in decrease of fat mass and increase of fat-free mass. In the TRT but no exercise condition, patients did not improve muscle function but decreased fat mass, increased fat-free mass, and upper body strength. Importantly, TRT plus progressive resistance training produced greater improvements in body composition than either intervention alone.

Glintborg et al. [59] studied effects of TRT and/or strength training (ST) on cardiovascular risk in hypogonadal males for 6 months. This double-blinded, placebo-controlled study found that only ST + placebo significantly decreased sCD36 levels. Only placebo group did not decrease fat mass during this period. Compared to TRT, six months of strength training reduced sCD36 levels suggesting decreased cardiovascular risk, possibly due to a reduction in central fat mass.

In a pilot randomized controlled trial by Cho and colleagues [60] when hypogonadal males were treated with combination of exercise and TRT, significantly better results in serum testosterone levels and symptoms of hypogonadism compared to TRT alone after 12 weeks of intervention were found. The levels of testosterone were significantly higher in the combination group ($p = 0.01$) In addition, these improvements were well-maintained in the combination group with continuous exercise even after cessation of TRT. After 20 weeks of intervention the group which used TRT and strength training kept the testosterone levels significantly higher ($p = 0.01$) compared to the group with TRT only. Consequently, it seems that exercise can augment the durability of response to TRT and it may be the solution to shorten the treatment duration with a lower risk from testosterone therapy [60]. There are some very promising results showing a great potential of exercise in hypogonadal patients. However, the above-mentioned

studies did not focus on possible physiological and metabolic mechanisms responsible for the positive effects of resistance training at circulating, cellular and molecular level. Up to date, there are no studies investigating the effects of strength training on the regulation of muscle mass and neuromuscular function at a cellular level in hypogonadal male patients.

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 (Fig. 2).

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

We declare that we have no significant competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

Figures

Figure 1: An algorithm for the diagnosis of hypogonadism (T - Total Testosterone) according to 7, 12-13. (Adapted from [3]).

Figure 2: Timeline of the ADAM study.

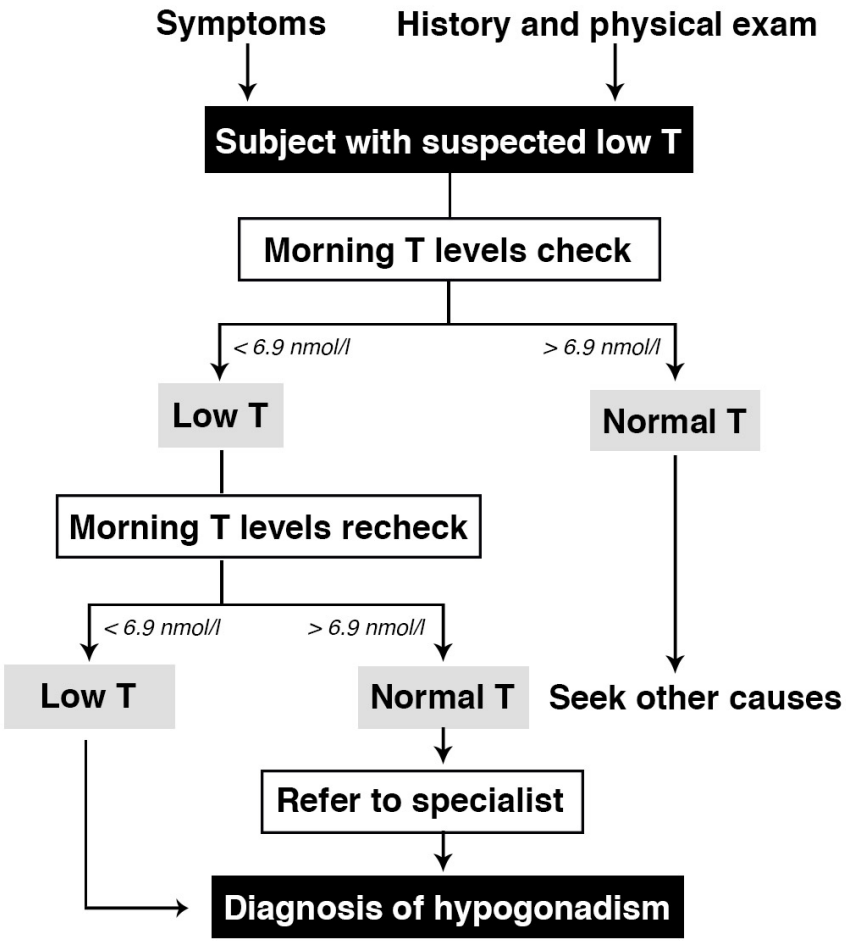

Figure 1: An algorithm for the diagnosis of hypogonadism (T - Total Testosterone) according to 7, 12-13. (Adapted from [3]).

99x108mm (300 x 300 DPI)

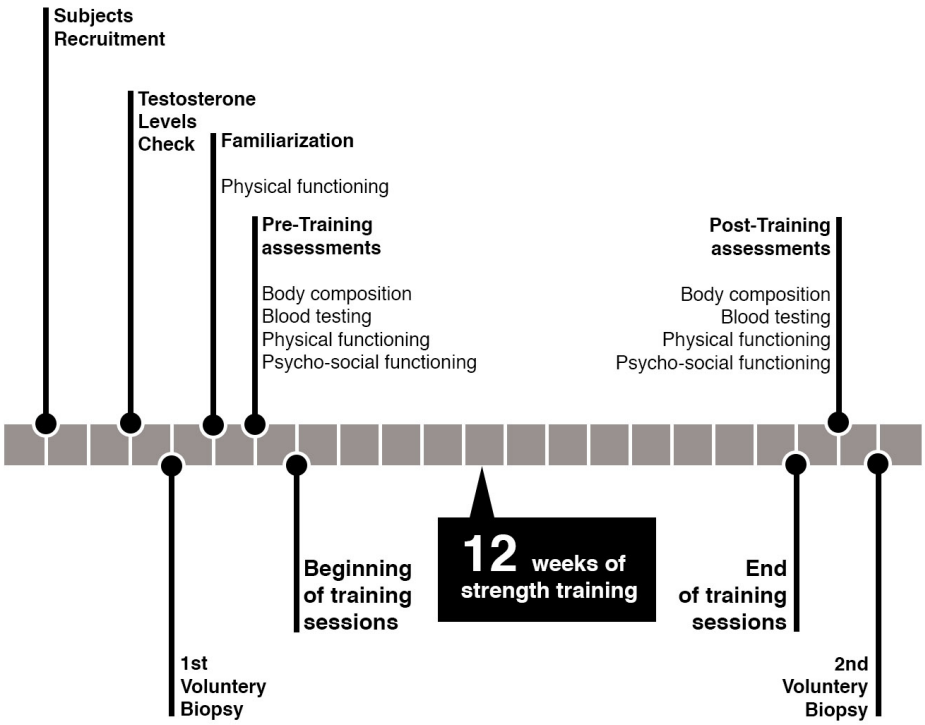

Figure 2: Timeline of the ADAM study.

119x99mm (300 x 300 DPI)

Additional file 1: Inclusion and exclusion criteria

Subject's Code: | | | | | | Subject's Date of Birth:

Subject's Name:

Inclusion criteria

1A. Newly diagnosed with ADAM syndrome ☐ Yes ☐ No
1B. Patient with ADAM syndrome on testosterone replacement therapy ☐ Yes ☐ No
2. 40 - 60 years of age ☐ Yes ☐ No
3. Capable of reading and writing Slovak ☐ Yes ☐ No
4. Treating urologist\endocrinologist has approved the subjects'participation ☐ Yes ☐ No
4. Lives within approximately 1 hour from Bratislava by car of public transportation ☐ Yes ☐ No
6. Written informed consent received ☐ Yes ☐ No

Exclusion criteria

1. Rutine resistance training with manuals ☐ Yes ☐ No
2. Medication for osteoporosis ☐ Yes ☐ No
3. Conditions that contraindicate exercise without adjusted actions ☐ Yes ☐ No
4. Mentally incompetent conditions ☐ Yes ☐ No
5. Conditions complicating ability to participate in a supervised training program ☐ Yes ☐ No
6. Abnormal DRV (digital rectal examination) ☐ Yes ☐ No
7. Serious system deases as
   a) cardiovascular deases ☐ Yes ☐ No
   b) liver and kidneys deases, ☐ Yes ☐ No
   c) diabetes mellius, ☐ Yes ☐ No
   d) oncological deases ☐ Yes ☐ No
   e) or other serious dease according to the judgment of the responsible physician. ☐ Yes ☐ No

Clinician's Signature: _____ Date:

Additional file 2: Outcomes, specific variables and assessments

Outcomes	Specific variables	Assessments
CLINICAL OUTCOMES		
Body composition		
	Lean Mass (LM) – primary outcome	DXA
	Fat Mass (FM)	DXA
	Total Body Mass (TBM)	DXA
	Body Mass Index (BMI)	Weight and height
The haematological and biochemical parameters	Haemoglobin(g/l), Hematocrits(ratio), Leucocytes (10^9/l), Thrombocytes (10^9/l), Glucose (mmol/l), Urea (mmol/l), Sodium (mmol/l), Potassium (mmol/l), Calcium (mmol/l), ALAT, Total Cholesterol (mmol/l), LDL Cholesterol (mmol/l), HDL Cholesterol (mmol/l), Triglyceride (mmol/l), Testosterone (nmol/l), Oestrogen (nmol/l), LH (nmol/l), FSH (nmol/l), SHBG (nmol/l), Albumin (g/l), Bilirubin (µmol/l), Total Protein (g/l), CRP(mg/l), Insulin (mIU/l), PSA (ug/l).	
Physical functioning		
	Muscle strength	10-m Usual Walk Test (s) 10-m Fast Walk Test (s) Maximal voluntary contraction (MVC) of isometric knee extension (Nm) Maximal voluntary contraction (MVC) of isometric knee flexion (Nm) 1RM on leg press (kg) Handgrip strength (kg)
	Cardio-respiratory fitness	The Single Stage Treadmill Walking Test (VO2max in ml.kg-1.min-1)
Psycho-social functioning		
	Symptoms of ADAM	Aging Males' Symptom (AMS) Scale
	HRQoL	SF-36
Muscle cellular outcomes		
Muscle fibre size	Muscle fibre cross sectional area (primary cellular outcome)	Cross sections of muscle biopsies
Regulators of muscle fibre size		
Number of myonuclei per muscle fibre		Number of myonuclei per muscle fibre
Number of satellite cells per muscle fibre		Cross sections of muscle biopsies

Number of satellite cells and myonuclei positive for androgen receptors		Cross sections of muscle biopsies

DXA - Dual-energy X-ray Absorptiometry, LDL - Low Density Lipoprotein, HDL - High Density Lipoprotein, LH - Luteinizing Hormone, FSH - Follicle Stimulating Hormone, SHBG - Sex Hormone-Binding Globulin, CRP - C- Reactive Protein, PSA - Prostate Specific Antigen, ADAM – androgen deficiency in aging male, HRQoL – Health-Related Quality of Life.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

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