**STUDY PROTOCOL PRO FORMA**

**Title**

Effects of exercise in controlling blood glucose in Type II Diabetes Mellitus patients.

**Aims**

The aim of this project is to compare the effects of eccentric versus concentric exercise on blood glucose level, functional physical fitness, and blood lipid profiles of Type II Diabetes Mellitus Patients.

**Hypotheses**

We hypothesise that the downhill treadmill walking is more effective in controlling blood glucose of type II Diabetics as compared to level or uphill treadmill walking.

**Background**

T2DM is a metabolic disease due to insulin resistance in the skeletal muscles and a beta cell defect that inhibits the increase in insulin secretion to compensate for insulin resistance. The prevalence of diabetes in Australia is considered an epidemic, such that 280 Australians develop diabetes each day, and is set to become the primary burden of disease in Australia in the next 5 years [1]. Within this context, T2DM represents 85-90% of all cases, and is getting diagnosed in progressively younger adults. The current annual cost of T2DM treatment to the nation is $14.6 billion and is projected to reach $30 billion in the next 12 years. Clearly, there is a need to reduce the cost of diabetes and its associated co-morbidities, especially when the incidence of T2DM could be reduced through effective interventions in 58% of cases [2].

The effects of physical exercise on T2DM are well documented, and physical exercise is one of the three cornerstones in the treatment of diabetes, along with diet and medication [3]. Several reviews (e.g [4]) and meta-analyses (e.g. [5]) report that increased physical exercise produce a significant improvement in glucose control in people with T2DM without adverse effects. Another systematic review and meta-analysis [6] compared resistance training and aerobic exercise, and concluded that there was no evidence that resistance exercise differs from aerobic exercise in impact on glucose control, cardiovascular risk markers or safety. Aerobic training and resistance training are both beneficial; however, a combination of the two is perhaps the optimal form of exercise for people with T2DM [7]. However, it has not been recognised that different muscle contraction modes during aerobic and resistance exercise can potentially make large differences in the effects of exercise training on T2DM.

Our daily physical activities and exercises consist of static (isometric), shortening (concentric) and lengthening (eccentric) muscle contractions, which are three typical modes of muscle contraction. Some activities and exercises such as descending stairs or slopes predominantly demand eccentric contractions, where contracting front thigh muscles are lengthened as part of controlling the rate of descent. We have been particularly interested in eccentric exercises, since they are less metabolically demanding, but stimulate skeletal muscles to increase muscle function and mass to a greater extent than concentric exercises in which muscles are predominantly shortened.

Several recent unpublished studies have tested the hypothesis that eccentric training (ET) of the knee extensors (KE) would improve health and fitness parameters more than concentric training (CT). A previous study investigated the hypothesis that descending stair walking (DSW), a typical eccentric exercise that we could perform in our daily activities without using equipment, would improve insulin sensitivity, lipid profiles, and functional physical fitness greater than ascending stair walking (ASW). The results supported the hypothesis that DSW would improve lipid profiles, insulin sensitivity and fitness of elderly overweight women better than ASS. It is important to note that DSW is less metabolically demanding than ASW. It is concluded that DSW can be used as an effective exercise intervention for less-fit individuals to improve their health and fitness.

Regular physical exercise is a key element in the prevention and management of type 2 diabetes mellitus (T2DM). Exercising regularly improves blood glucose control and can prevent or delay T2DM and its complications, along with positively affecting lipids, blood pressure, cardiovascular events, mortality, and quality of life. However, most people with T2DM are not active and show poor adherence to exercise. Previous prospective cohort and cross-sectional observational studies that assessed physical activity with questionnaires showed that higher levels of physical activity are associated with decreased risk for T2DM. Observational studies have reported that greater fitness is associated with a reduced risk of developing T2DM, even if only moderate-intensity exercise is undertaken [8]. Regular physical activity improves blood glucose control and positively affects lipids, blood pressure, cardiovascular events, mortality, and quality of life as well [8]. With possible increase in glycogen synthase activity and glucose transporter 4 protein expression, regular PA plays an important role in the glycaemic control, which can reduce HbA1c.

Despite the clear evidence that exercise is a key element in controlling and managing T2DM, individuals with diabetes are among the least likely to engage in regular exercise, and the adherence to exercise is surprisingly poor. Previous studies have shown that most adults with T2DM or at highest risk for developing it do not engage in regular physical activity, the rate of participation is significantly below national norms. [9]. In a survey of "the situation of self-management in Chinese patients with T2DM" from Chinese Diabetes Society in 2010 reported that only 35.2% of patients with T2DM remained physically active at recommended levels of physical activity. In the other survey of adults aged 55 years with T2DM, 55% of respondents reported no weekly exercise. [10].

The purpose of this study is to compare the effects of uphill, downhill and level treadmill walking on health outcomes and functional fitness in a group of type II diabetics. No previous study has compared uphill vs downhill treadmill walking and its effect on blood glucose in T2DM. This study will be the first of its kind and will open avenues for future research.

**Methods**

Design

This is a randomised controlled trial.

Intervention

This study will involve a comparison of predominately eccentric (Downhill walking) versus predominantly concentric (Uphill walking) and mixed eccentric and concentric (Level walking) endurance exercise. Participants willing to participate in the study will be screened for balance by Berg Balance Scale. Patients having good balance and those falling under low fall risk will be included in the study and then will be divided into three groups. Participants in one group will perform downhill walking, participants in the second group will perform uphill treadmill walking and participants in the third group will perform level walking, twice a week for 12 weeks with 2-3 days between sessions. Exercise sessions will commence at 5 minutes duration at the same comfortable walking pace and progress to 30-minutes sessions by the last week. Participant progression will be individualised according to participants capabilities as determined by the baseline physical function assessments and rating of perceived exertion (RPE) within each exercise session. RPE will be maintained under 11 (Fairly Light) on the 6-20 point scale for all sessions. Adherence to exercise during the exercise intervention will be measured via attendance sheets at training sessions and records of the amount of time walked and the gradient for each session. All the outcome measures will be assessed at baseline and after the completion of the 12-week exercise programme. At the completion of the endpoint testing, participants will be asked to come for an additional session in which metabolic cost of submaximal uphill, downhill and level treadmill walking will be calculated through gas analysis of expired air collected at 15s intervals using a metabolic cart (Parvo TrueOne; Parvomedics, UT, USA). The collection of this data will allow a comparison of the energy costs associated with walking at each of the gradients and enable outcome data to be corrected for the energy cost of each exercise intervention.

Setting

The study will be conducted at Exercise clinic, School of Health Sciences at University of Tasmania, Launceston

Participants

Potential participants will be recruited through flyers placed at GP clinics, diabetes clinics at the Launceston General Hospital and support groups such as Diabetes Tasmania.

Inclusion Criteria: Patients having following characteristics will be included.

Diabetic type II patients at least 18 years of age not currently engaging in regular exercise (not currently meeting Australian physical activity recommendations) [11]

Exclusion Criteria: Patients having following characteristics will be excluded.

* Diabetic type II patients involved in regular physical exercise
* Patients suffering from progressive neurological diseases.
* Patients unable to provide informed consent
* Patients with poor balance (high fall risk)

All interested potential participants will be provided with further information by a researcher who is a physiotherapist, working under an accredited exercise physiologist. This researcher will be involved in additional clinically relevant screening of potential participants for conditions that could be made worse by exercise.

Sample size and justification

Patients recruited in the study will be randomly divided into three groups through block randomisation technique. A sample size calculation was performed using STATA based on a previously reported change in HbA1C in eccentric (mean ± SD: -6.0±3.0%) and concentric training (mean ± SD: -2.83 ±2.17%) groups over a similar length intervention [12]. With an alpha of 0.8 and α-level of 0.05 corrected to 0.017 to allow for multiple comparisons, a total of 42 participants (14 per group) is necessary. To deal with an expected withdrawal rate of 15% during the study, 48 participants will be recruited. Patients will randomly be divided into three groups. 16 patients will be in each group.

Recruitment

Patients will be recruited in the study by the principal investigator through General Practitioners and other physicians, support organization such as Diabetes Tasmania and Out-patient department of Launceston General Hospital. Various advertising strategies, such as distributing pamphlets to university personnel and residents of nearby communities and posting recruiting posters in outpatient departments at university hospitals, will be used to recruit participants.

Measures

The primary outcome measure of this study will be insulin sensitivity and various other secondary outcome measures will be accessed.

1. Insulin sensitivity and lipid profiles: The primary outcome measure of this study is HBA1c and OGTT. HbA1c will be measured via DCA Vantage point of care analyser (Siemens Healthcare, Tarrytown, NY). The DCA Vantage analyser has previously been demonstrated to be a reliable and valid method for determining HbA1c [13]. In the OGTT, after the baseline blood sample is taken as described above, each participant will have a 75-g standard glucose drink, and blood samples will be taken at 30, 60, 90, and 120 min after the ingestion. Blood glucose will be measured using a Hemocue Point of Care device (Hemocue Glucose 201; Hemocue AB; Angelholm, Sweden), a reliable and valid device for measuring blood glucose [14], and the area under the curve from the baseline to 30, 60, 90 and 120 min will be calculated. Finger prick capillary blood samples taken by a trained professional will be used to measure HbA1c and blood glucose. Blood lipid profiles will consist of serum tricylglycerols (TG), total cholesterol (TC), high- (HDLC), low-density lipoprotein cholesterol (LDHC), apolipoprotein A1 (Apo-A1) and B (Apo-B), and for measuring these a venous blood sample of 10 ml will be taken by a trained professional at university lab.
2. Muscle function and upper thigh circumference: For the study, maximal voluntary contraction (MVC) strength of the knee extensors and flexors will be measured before and after training by a Humac isokinetic dynamometer. For determining maximal voluntary contraction, Humac isokinetic dynamometer has previously been demonstrated to be a reliable and valid tool [15]. For upper thigh circumference, each participant will stand with the feet being approximately 10 cm apart and body weight evenly distributed between feet, and the measurement will be taken at the mid-point between the greater trochanter and epicondyle of femur of the exercised leg using a Gulick tape.
3. Heart rate, blood pressure: Each participant will rest in supine position on a padded table for at least 10 min before the measurements. HR, brachial blood pressure, blood flow and arterial stiffness are taken in a sequence. Resting HR and brachial blood pressure will be measured by the Sphygmocor XCEL central blood pressure system (SphygmoCor XCEL System V1, AtCor Medical, Sydney, Australia).
4. Arterial Stiffness: Arterial stiffness will be assessed by central (carotid-femoral) Pulse Wave Velocity (PWV) which will be measured using non-invasive and simultaneous measures of the arterial waveforms at the carotid artery (using applanation tonometry, Sphygmocor T-03C Tonometer, Sphygmocor XCEL, Sydney, Australia) and the femoral artery (using a pneumatic cuff to determine pulsations) connected to a physiological signalling processing system (SphygmoCor XCEL System V1, AtCor Medical, Sydney, Australia) as previously described [16]. Peripheral (carotid-brachial) PWV will be assessed as described above from waveforms acquired at the common carotid artery and a pneumatic cuff at the brachial artery. Central blood pressures will be estimated by pulse wave analysis using a customised system and software (SphygmoCor XCEL V1.3, AtCor Medical, Sydney, Australia) and which involves a pneumatic cuff placed on the dominant arm that determines key parameters of the central aortic pressure waveform via the pulsations of the blood, as described previously [16].
5. Biomarkers: Evidence exists that regular exercise offers protection against chronic disorders such as cardiovascular diseases and type 2 diabetes. Muscle fibres produce and release the cytokines IL-6 and irisin into the circulation during exercise [17]. To access the beneficial effects of irisin and IL6, released due to exercise on controlling blood glucose, they will be measured by routine enzyme-linked immunosorbent assay (ELISA). Creatine kinase is produced in the body because of muscle damage. To evaluate which type of exercise is more damaging for muscles, CK will also be measured by ELISA. The venous blood sample of 10 ml taken by venepuncture for measurement of lipid profiles will also be used to measure these biomarkers. ELISA kits (LifeSpan Biosciences, Inc) will be used to measure Irisin, IL-6 and CK as per manufacturers instructions. These biomarkers will be measured initially before the start of exercise and then after the 12 weeks exercise program.
6. Functional physical fitness tests: physical fitness of individuals will be tested through 6 min walk, timed up and go test and the 30 second Sit-to-Stand Test (30sSTS) in this study. The participants will perform these tests initially before starting the exercise and then at the end of the 12 weeks. The 6 Minute Walk Test (6MWT) is a sub-maximal exercise test used to assess aerobic capacity and endurance. The distance covered over a time of 6 minutes is used as the outcome by which to compare changes in performance capacity. The 6MWT will be performed in a 20-m corridor located in the School of health Sciences Building at University of Tasmania; tape will be placed every 2 m. Participants will be asked to walk the longest distance possible in 6 minutes by walking continuously the 20 m indicated on the floor, turning around at the final mark without stopping, and covering as much ground as possible. The standardized order given to the participants will be, “Walk as far as possible for 6 minutes, but don't run or jog.” The distance covered (in meters) will be recorded at the end of the test. 6 in walk test has good validity and reliability. [18]. The Timed Up and Go test (TUG) is a simple test used to assess a person's mobility and requires both static and dynamic balance. In this test, time is calculated that a person takes to rise from a chair, walk three meters, turn around, walk back to the chair, and sit down. This test will be carried out in lab in School of Health Sciences building. the Timed up and Go test has excellent interrater (intraclass correlation coefficient [ICC] = .99) and intrarater reliability (ICC = .99)[19]. To evaluate lower limb strength and the ability to perform activities of daily living the 30 second Sit to Stand Test will be performed. In this test the participant sits with their back against the chair and the arms are folded across the chest while the feet remain on the floor throughout the test. The participant is asked to stand and sit and no of repetitions are recorded which the participant performs in 30 second time. [20]
7. Metabolic Cost: Energy expenditure will be measured via gas analysis on two separate occasions. The first time will be during the prescribed exercise bout during the first week of the intervention at the initial gradient (-4%, level, or +4% depending on group allocation). Each participant will be invited back at the end of the study to complete a walking bout with 4 minutes of walking at each gradient used across the three intervention protocols (4, 8, 12, and 16% uphill and downhill and one level walking; 36 minutes in total). Expired gas will be collected during the fourth minute of walking at each gradient and will be analysed immediately after the end of the trial. The energy cost of the initial gradient in the first week will be compared against the energy cost of the same gradient in the final week to compare for any changes in metabolic efficiency over the duration of the study. Metabolic data that is obtained will be used to generate relative energy expenditure curves for a diabetic population at different walking gradients similar to those that have previously been generated in healthy young adults [21]. The data that is obtained will also allow any changes in the major outcomes to be compared between groups based on energy expended during the intervention.

Data Collection

A consort diagram is provided to outline timepoints. After initial recruitment in the study the participants will be randomly allocated to three groups via block randomisation with equalisation between groups every 6 or 9 participants by an independent researcher not involved in assessment or delivery of the intervention using a computer-generated sequence of numbers. Group allocation into an uphill treadmill exercise group (n=13), downhill treadmill exercise (n=13) group and level walking group (n=13) will be stored in sealed opaque envelopes and opened in front of the participant after baseline assessment. All the groups will perform exercise twice a week for 12 weeks. 12 weeks has been chosen as a suitable time-frame to develop habitual exercise patterns.

Demographic data including age, gender, weight, height, time since diabetes, medications including doses, physical activity levels, occupation, socioeconomic status, educational level, time since diagnosis, co-morbidities will be collected through questionnaire from all the participants at the start of the study. Participants will be asked to report any changes in medications including doses and/or physical activity unrelated to the intervention over the study duration. Outcomes measures will be assessed by the primary investigator at the start of the study and then again after the completion of 12 weeks exercise programme.

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| Task | Start | Finish |
| Recruitment | july 19 | Sep 19 |
| Baseline Data collection (test 1) | Sep 19 | Oct 19 |
| Intervention | Oct 19 | Dec 19 |
| Follow up data collection (test 2) | Jan 2020 | Jan 2020 |
| Data analysis | Feb 2020 | Feb 2020 |

Quality control and feasibility

Quality Data Collection. All outcome measures are reliable and valid measures that are used widely in clinical practice. To ensure reliable and consistent test procedures clear and concise standardised test protocols will be written down and will be conducted by the principal investigator only.

Feasibility. Recruitment posters will be distributed to General Practices, Specialists clinics and support organisations such as Diabetes Tasmania and staff at all sites will be encouraged to promote the study to potentially eligible patients. Given the large number of potentially eligible participants that will be reached, and the small number of participants required for the study we believe that we can realistically recruit the required numbers within a three-month timeframe.

Data analysis

Data will be assessed (intention-to-treat and per-protocol) through STATA statistical software (STATA SE v15). Between group differences will be analysed using a linear mixed models’ assessment to determine differences between groups (uphill treadmill exercise vs downhill treadmill exercise vs level walking treadmill exercise) and between time points (TEST 1 and TEST 2). If the assumptions of linear regression are not met the analysis will be conducted using mixed effects logistic regression. Potential confounders including baseline physical activity, adherence to intervention (including walking duration) will be adjusted for in data analysis.

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