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FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN
master in de biomedische wetenschappen

Masterproef

Prevention of Type II diabetes mellitus through lifestyle intervention in predisposed obese adults

Promotor :
dr. TANJA ADAM

Copromotor :
Prof.dr. MARGRIET S. WESTERTERP-PLATENGA

Eline Vandeput
Scriptie ingediend tot het behalen van de graad van master in de biomedische wetenschappen

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Content

1 List of abbreviations

BMI	Body mass index
CID	Clinical investigation day
FFA	Free fatty acids
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
OGTT	Oral glucose tolerance test
T2DM	Type 2 diabetes mellitus
VO2max	Maximal oxygen consumption
VO2	Oxygen consumption
RCT	Randomized, controlled, multicentre trial
BW	Bodyweight
FM	Fat mass
FFM	Fat free mass
BF%	Body fat percentage
BAQ	Baecke questionnaires
LCD	Low calorie diet
W:H ratio	Waist to hip ratio
MI	Moderate intensity
HI	High intensity
SD	Standard deviation
LI	Low intensity

2 Acknowledgements

During the last 5 years, my education at Hasselt University helped me to become a real scientist with the knowledge and the skills necessary to help in conducting good research. For my senior internship, I got the opportunity to do research at Maastricht University. This opportunity helped me grow even further and I really enjoyed my time in the Netherlands. I worked here at the department of human biology and performed research in order to prevent type II diabetes mellitus in overweight or obese adults. This project was completely different from my other internships and therefore it helped me to develop new skills and prepare me for future challenges.

During this project I learned how important teamwork and communication is, especially when you are doing research at this level. You need to communicate with your supervisors, but also with the subjects taking part in the study.

I want to take the time to thank the people who helped me develop these different skills. First of all, I want to thank dr. Tanja Adam for helping me with my thesis and especially for her help with the statistical part. I want to thank her for her time and support. I could always pass by her office when I needed help or when I got stuck with my thesis. Next, I wanted to thank prof. dr. Margriet S. Westerterp-Platenga for her help with my thesis and to give me the opportunity to be part of the PREVIEW team. I also want to thank my daily supervisor Mathijs Drummen and the research assistant of the PREVIEW study, Nuria Esteban Rosique, they helped me to develop the skills necessary for this study. We worked together every day and they were always available to help me when this was necessary.

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3 Abstract

Type II diabetes mellitus (T2DM) is a chronic disease that is characterized by high blood glucose levels and insulin resistance. The incidence of this disease is rising fast, in part due to the increased prevalence of obesity. Lifestyle intervention can contribute to weight loss and the prevention of T2DM. We hypothesize that high intensity (HI) physical activity significantly improves physical fitness and glucose tolerance compared to moderate intensity (MI) physical activity. MI physical activity however will be more effective for the improvement of the body composition. Three objectives are formulated to investigate the effect of weight loss and subsequent physical activity on body composition (1), glucose tolerance (2) and physical fitness (3).

The study was conducted as part of a randomized, controlled, multicentre trial. Measurements were performed in overweight or obese pre-diabetic adults, who were randomly assigned to the HI (n=15) or MI physical activity group (n=18). Anthropometric measurements were performed with the use of a BodPod and an oral glucose tolerance test (OGTT) was used to measure glucose tolerance. Physical fitness (VO2max) was measured with an incremental ergometer test. Baecke questionnaires and accelerometers were used to evaluate the physical activity level of the participants. Since no differences in physical activity parameters were observed between the MI and HI group, the anthropometric and blood glucose data have been taken together for the whole group.

All body composition parameters significantly decreased between CID1 and CID2 (p=0,000 for all parameters), and between CID1 and CID3 (p=0,000 for all parameters; except for fat free mass, p=0,028). Fat free mass and body fat percentage significantly increased again between CID2 and CID3 (p=0,000 and p=0,005 respectively). Waist to hip ratio significantly decreased between CID1 and CID2 (p=0,011). In addition, an OGTT was performed and fasted blood glucose concentrations significantly decreased between CID1 and CID3 (p=0,000). Finally, correlation coefficients were computed and significant negative correlations were found between VO2max and bodyweight (r=-0,384, p=0,027), VO2max and body fat percentage (r=-0,560, p=0,001), VO2max and fat mass (r=-0,417, p=0,016), and VO2max and BMI (r=-0,450, p=0,009). A significant positive correlation was found between FFM and sport index (r=0,360, p=0,039).

Based on these results, it can be concluded that physical activity improves body composition and glucose tolerance. However, a drawback of this study is that the physical activity intensity was not strictly controlled which should be done in a follow-up study to determine the effect of physical activity intensity on these parameters instead of the effect of overall physical activity.

4 Samenvatting

Type II diabetes mellitus (T2DM) is een chronische ziekte die gekenmerkt wordt door een hoge glucose concentraties en insuline gevoeligheid. De incidentie van T2DM stijgt snel, deels door de stijgende prevalentie van obesitas. Verandering van de levensstijl kan bijdragen aan gewichtsverlies en op die manier bijdragen aan de preventie van T2DM. We verwachten dat beweging met een hoge intensiteit (HI) zorgt voor een significante verbetering van de fysieke fitheid en de glucose tolerantie in vergelijking met beweging met een gematigde intensiteit (MI). MI zorgt dan weer voor de verbetering van de lichaamssamenstelling. Drie doelstellingen worden geformuleerd om het effect van gewichtsverlies and fysieke activiteit op lichaamssamenstelling (1), glucose tolerantie (2) en fysiek fitheid te onderzoeken.

De studie werd uitgevoerd als deel van een gerandomiseerd, gecontroleerd, multicenter onderzoek. Metingen werden uitgevoerd in volwassen prediabeten met overgewicht of obesitas. Deelnemers werden willekeurig toegewezen aan de groep met HI beweging (n=15) of MI beweging (n=18). Antropometrische metingen werden uitgevoerd met behulp van een BodPod en een orale glucose tolerantie test (OGTT) werd gebruikt om glucose tolerantie te evalueren. Fysieke fitheid (VO2max) werd gemeten met behulp van een incrementele ergometer test. Baecke vragenlijsten en accelerometers werden gebruikt om de fysieke activiteit van de deelnemers te evalueren. Aangezien er geen verschillen waren in intensiteit tussen de MI en HI groep, zullen beide groepen samen genomen worden voor verdere analyses.

Alle parameters van lichaamssamenstelling daalde significant tussen CID1 en CID2 (p=0,000 voor alle parameters), en tussen CID1 en CID3 (p=0,000 voor alle parameters; behalve voor vetvrije massa, p=0,028). Vetvrije massa en vetpercentage stegen significant tussen CID2 en CID3 (p=0,000 en p=0,005 respectievelijk). Taille tot heup ratio daalde enkel significant tussen CID1 en CID2 (p=0,011). De nuchtere bloed glucose concentratie daalde significant tussen CID1 en CID3 (p=0,000). Correlatie coëfficiënten werden ook berekend en er werden significant negatieve correlaties gevonden tussen VO2max en gewicht (r=-0,384, p=0,027), VO2max en vetpercentage (r=-0,560, p=0,001), VO2max en vetmassa (r=-0,417, p=0,016), VO2max en BMI (r=-0,450, p=0,009). Een significant positieve correlatie werd gevonden tussen vetvrije massa en sport index (r=0,360, p=0,039).

Er kan geconcludeerd worden dat fysieke activiteit de lichaamssamenstelling en de glucose tolerantie verbeterd. Een nadeel van de studie is echter dat de beweging niet gecontroleerd werd. Dit zou moeten gebeuren in een follow-up studie om het effect van bewegingsintensiteit op de verschillende parameters te kunnen onderzoeken.

5 Introduction

5.1 Obesity

Obesity remains a serious worldwide health problem and its prevalence is rising fast. In order to classify obesity, the body mass index (BMI, calculated as weight in kilograms divided by height in meters squared) is used. Persons with a BMI higher than 30 kg/m^2 are considered obese, but adults are already considered overweight when they have a BMI between 25 and 30 kg/m^2 (1). However, BMI is not the only measure to evaluate obesity; waist circumference can also be used. In this case abdominal obesity in women is characterized by a waist circumference of more than 88 cm and more than 102 cm in men (2). A third measure for obesity is body fat percentage. In this case, obesity is defined as a body fat percentage of ≥ 25% for men and ≥ 35% for women (3).

Obesity can be described as a complex multifactorial disease, which develops through an interaction of genetic factors, physiological issues, and the environment (4). In general, obesity results from a positive energy balance, which means that energy intake exceeds energy expenditure, over a long period of time (5). Being overweight or obese increases the likelihood of developing different adverse metabolic effects, like for example insulin resistance (6) which means that there is a decreased response of the tissues to insulin. People that are insulin resistant are predisposed for the development of T2DM (7). In addition, obesity leads to a high mortality and morbidity and increases the risk for the development of several other serious diseases, like coronary heart disease, stroke, respiratory problems and several forms of cancer (4).

5.2 Type II diabetes mellitus

Type 2 diabetes mellitus (T2DM) is the fastest growing chronic disease and it is characterized by high blood glucose levels and insulin resistance (8). T2DM is the most common form of diabetes (9) and it is a major cause of death. The World Health Organisation believes that the total deaths caused by this disease, will rise with 50% in the next 10 years. This form of diabetes is due to a combination of genetic and environmental factors. However, the environmental factors, like smoking, excessive caloric intake, decreased physical activity and heavy alcohol consumption play a bigger role in the development of T2DM (9). Therefore, the growth of T2DM can be related to the increased prevalence of obesity which is caused by a sedentary lifestyle and physical inactivity (8).

Prior to T2DM, these people suffer from pre-diabetes. The prevalence of pre-diabetes is three times higher than that of T2DM. Every year, 5% to 10% of the pre-diabetic people become diabetic and if this trend continuous, 1 in 3 adults will have T2DM by 2050. Pre-

diabetes is characterized by a fasting blood glucose level between 5.6 mmol/L (100 mg/dL) and 6.9 mmol/L (125 mg/dL), and/or a blood glucose level between 7.8 mmol/L (140 mg/dL) and 11.0 mmol/L (199 mg/dL) after a two-hour oral glucose tolerance test (OGTT) (10).

The current treatment options can prevent some of the complications of T2DM but these methods are not capable to restore normal insulin sensitivity and blood glucose levels. Therefore, prevention of T2DM and especially pre-diabetes is preferable. A possible option to prevent T2DM in pre-diabetic adults can be lifestyle intervention.

5.3 Relation between obesity and type II diabetes mellitus

Research has shown that the relative risk of getting T2DM increases exponentially with an increased BMI. The relative risk of developing T2DM is already 2,67 for a BMI between 23 and 24,9 kg/m². This means that a BMI of 23 kg/m^2 would already double the risk of getting T2DM compared to a BMI lower than 23 kg/m². The relationship between obesity and T2DM is in general stronger in women than in men (1).

Therefore, diet and physical activity interventions are necessary to lose weight and prevent weight regain (5). In order to lose weight it is important to create a negative energy balance. Hereby, sustained satiety is important to maintain this negative energy balance and to lose weight (5, 11). Next to this, a sustained basal and total energy expenditure is also important and can be achieved by sparing fat free mass (FFM). A diet with a higher protein content allows the maintenance of this negative energy balance by preservation of FFM (12, 13).

Body composition may improve even more (increase of FFM and decrease of FM) when weight loss is achieved through a combination of a protein diet with exercise. However, there is no consensus about which type of exercise will have the best effects on weight loss and FFM preservation (14). Some studies show that exercise-induced weight loss increases the maximal oxygen uptake (VO2max), and thus the physical fitness. In contrast, a low calorie diet (LCD) alone leads to a decrease in physical fitness (15). Therefore, a combination of diet and exercise intervention is important in order to increase or maintain physical fitness.

5.4 Physical activity

As mentioned before, physical activity is an important component in the prevention of T2DM. Even the easiest form of physical activity, like walking, can already reduce the risk of T2DM, therefore a combination of physical activity and diet intervention will give the most successful results (16, 17).

In order to perform physical activity, fuel needs to be mobilized from reserves within the muscle itself but also from extra muscular fuel depots like the liver. The amount and type of fuel that is used, will be determined by the fuels available from the diet, the duration and the intensity of the activity (16, 18).

5.4.1 Moderate intensity physical activity

During moderate intensity (MI) physical activity, the body can keep up with the demand for oxygen. This means that the activity is aerobic and with the availability of oxygen, the muscle can derive its energy from the oxidation of both glucose and fatty acids. At the start of a MI exercise session, the energy is derived from glucose and half from free fatty acids (FFA). In order to keep up with the demand for glucose, liver glycogen is converted into glucose and released into the bloodstream were it is available for the muscle to use. Next to this source of glucose, muscles can also use their own glycogen stores as a fuel source for their work (18).

When the duration of this moderate exercise session exceeds one hour or more, the glycogen stores of the liver become depleted, which means that there will no longer be a release of glucose from the liver into the bloodstream. In addition, the hormone epinephrine will be released from the adrenal medullae as a result of sympathetic stimulation, which leads to a breakdown of the triglyceride stores in the fat cells, allowing the release of FFA into the bloodstream. This leads to an increased use of FFA as an energy source for the muscles and a decrease in the use of glucose. Towards the end of the exercise session, the largest amount of energy will be derived from FFA (80%) (18).

5.4.2 High intensity physical activity

A different metabolic situation takes place during a high intensity (HI) physical activity session. In this case, the rate of exercise often exceeds the capacity of the body to provide enough oxygen for aerobic activity. This means that the muscles can only use glucose as an energy source because it is the only fuel that can be used anaerobically, energy can be produced from glucose without the simultaneous use of oxygen. Another advantage of the use of glucose is that the energy is transferred more rapidly compared to the use of FFA. Glucose will first be supplied by the glycogen stores of the muscle. When these glycogen stores become depleted, blood glucose becomes the major source of energy. Depletion of muscle glycogen also leads to muscle fatigue which decreases the exercise capacity. When muscle and liver glycogen stores become depleted, the blood glucose level decreases which may lead to hypoglycaemia (18).

5.5 The effect of physical activity and caloric restriction on body composition, physical fitness and glucose tolerance

In order to change or effect the body composition, glucose tolerance and physical fitness, a combination of both exercise and diet intervention is one of the best options. Caloric restriction by itself for example, would lead to a decrease in muscle mass (FFM) and a change in FFM may possibly lead to a decrease of the physical fitness. A combination of caloric restriction and physical activity can diminish the decrease of FFM and thereby preserve the physical fitness (15).

5.5.1 Body composition

An increase of exercise duration, intensity and frequency in combination with a diet intervention will lead to changes in body composition. The loss of fat mass (FM) is the major outcome of interest because fat can have harmful effects dependent on its location, amount and time of fat deposition. In adults, excess body fat is associated with different diseases, like T2DM and hypertension. In addition, excess FM is also associated with an increased mortality and morbidity (19).

Diet intervention alone reduces the bodyweight but often this weight loss is not equal to the loss in FM because in most cases it also leads to a decrease of the FFM (which includes bone and muscles). Therefore, it is important to look at the change in FM and FFM instead of only looking at the overall weight loss (19).

5.5.2 Physical fitness

The increase of inactivity that often accompanies obesity, is part of a tendency towards dysfunction or even in some cases an increased morbidity because a low amount or lack of physical activity leads to physical weakness. Therefore, regular physical activity is very important to overcome this physical weakness and ultimately increase the physical fitness (20).

The beneficial effects caused by regular physical activity depend on intensity or amount of work performed during one exercise session. This is also demonstrated by the observation that physical fitness, measured as VO2max is a major predictor for all-cause mortality (21, 22). Research showed that metabolically healthy obese people have a higher physical fitness compared to metabolically unhealthy (based on glucose concentrations, fasting triglycerides, HDL-cholesterol and LDL-cholesterol) obese people (20, 23).

5.5.3 Glucose intolerance

Exercise can be part of a good treatment for a decreased glucose tolerance (24). A study of Ross R. et al showed that 200 minutes of vigorous exercise per week could lead to a 9% improvement of the glucose tolerance when this is combined with a 5% to 6% weight reduction (25). The effect of exercise on the glucose tolerance is mostly attributed to the most recent session and not to the accumulated effect of different exercise sessions or physical fitness. One single HI exercise session is enough to improve glucose tolerance. However, this is only a short-term effect and therefore regular physical activity is encouraged when exercise is used for the improvement of insulin sensitivity (24, 26). Regarding exercise intensity, MI physical activity has no beneficial effects on the glucose tolerance. In contrast, HI physical activity can improve glucose tolerance in obese adults (25).

5.6 Research plan and objectives

In order to investigate which type of exercise intensity improves body composition, physical fitness and glucose tolerance, a diet intervention in combination with an exercise intervention will be performed in this studi. The study population consists of predisposed, pre-diabetic obese adults who are glucose intolerant and therefore have a high risk of insulin resistance. It is believed that simply losing weight can reverse this glucose intolerance. In order to lose weight, the participants will undergo a very LCD (810 kcal/day) during a weight loss period of eight weeks. This period is followed by a weight maintenance period. During this period, the participants will be randomly assigned to one of the two study groups; a moderate protein diet and HI physical activity or a moderate protein diet and MI physical activity (Figure 1). The moderate protein diet consists of 15% proteins, 55% carbohydrates and a glycemic index of more than 65%. These dietary guidelines have been chosen because they are healthy and supportive for weight maintenance.

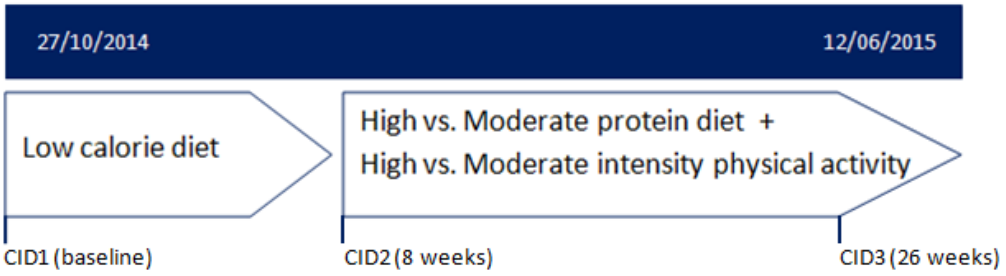

Figure 1. Study overview. Participants will first follow a LCD during a weight loss period of 8 weeks and this period is followed by the intervention period during which participants follow a moderate protein diet in combination with moderate or high intensity physical activity.

The research question of this study is whether HI physical activity has a different effect on physical fitness, glucose tolerance and body composition compared to MI physical activity? We hypothesize that HI physical activity significantly improves the physical fitness and glucose intolerance compared to MI physical activity, but MI physical activity is more effective for the improvement of the body composition.

Three objectives are formulated for this study in which exercise intervention is applied after a calorie-restriction/weight loss period. The first objective is to investigate the effect of weight loss and subsequent physical activity on body composition. Body composition measurements will be done by BodPod. This technique is one of the least invasive techniques to test body composition and it uses air displacement to measure body volume and to evaluate the possible preservation of the FFM at different stages of the study. The bodyweight and height of the participants is measured at different time points to calculate their BMI. While the subjects are in a fasting state in the morning, their hip, waist and thigh circumference will also be measured at different time points throughout the study.

The second objective is to investigate the effect of weight loss and subsequent physical activity on glucose metabolism. An OGTT will be performed on every subject. At the start of the OGTT, the participant must be in a fasted state. During this test, each participant will take an oral load of 82,5 g glucose. After this glucose intake, the participant is not allowed to drink or eat anything. Blood samples will be obtained during the OGTT for the measurement of glucose at baseline and 120 minutes after the glucose intake in order to evaluate glucose tolerance and identify possible new cases of T2DM.

The third objective is to investigate the effect of weight loss and subsequent physical activity on physical fitness. Physical fitness (VO2max) will be investigated in a subgroup of forty-three adults by using an incremental ergometer test, which measures respiratory gases. This test is submaximal up to an estimated 85% of VO2max and the test will be performed at baseline and after 26 weeks.

6 Materials and methods

This study was conducted as part of the PREVIEW-intervention study, a randomized, controlled, multicentre trial (RCT). The PREVIEW study is a lifestyle intervention study approved by the Medical Ethics Committee of the Maastricht University Medical Centre. All participants gave written informed consent.

6.1 Subjects

Based on different exclusion and inclusion criteria (Supplementary information, p. 29) and on information obtained after an interview and several questionnaires, forty three predisposed, pre-diabetic obese adults (male and female) were recruited. A paper version of the research protocol was handed to them. The study was also explained orally on the screening day and informed consent was obtained. Ten of the participants who started dropped out, leaving a total of thirty three participants. The most important reason for drop out was the inability of the participants to reach the 8% weight loss after the LCD. The participants were overweight or obese (BMI > 25 kg/m^2) and aged between 25 and 70 years.

Assumed difference between the groups moderate and higher intensity physical activity is 3 ml/kg/min (assuming an increase of 3 ml in the moderate and 6 ml in the vigorous group) (27), with a standard deviation of 5 ml, alpha = 0.05, beta = 0.20 and effect size=0.25. This leads to a total sample size of thirty four participants. The power analysis was performed with G power version 3.1 for Windows by conducting an F-test, ANOVA repeated measures within factors (28).

6.2 Dietary intervention

In order to lose weight, the participants will undergo a very LCD (±810 kcal/day) during a weight loss period of eight weeks (provided by Cambridge Weight Plan). The weight loss period is followed by a weight maintenance period. Participants can only take part in this second part of the study when they lost 8% of their bodyweight. During this weight maintenance period, the participants will be randomly assigned to one of the two study groups (Table 1). All participants will follow a moderate protein diet that consists of 15% proteins, 55% carbohydrates and a glycemic index of more than 65%. Diet is not supervised, but recommendations will be given in order to reach the appropriate amount of proteins, carbohydrates and the correct glycemic index.

Table 1. The two study groups.

	Moderate protein diet
High-intensity physical activity	Group 1: MP-HI
Moderate intensity physical activity	Group 2: MP-MI

6.3 Physical activity intervention

Together with the dietary intervention, participants will also undergo a physical activity intervention (Table 1). The first possible physical activity intervention is a HI physical activity intervention which is characterized by a heart rate of 76-90% of the maximal heart rate or a Metabolic equivalent of a Task (MET) value higher than six. The second intervention is a MI physical activity intervention which is characterized by a heart rate of 60-75% of the maximal heart rate or a MET value between three and six. Physical activity is not supervised. Recommendations for physical activity duration are 150 min/week of MI physical activity and 75 min/week of HI physical activity.

6.4 Anthropometrics and body composition

The height (m) of the participants was measured using a wall-mounted stadiometer (Seca-stadiometer) and bodyweight (kg) was measured using a calibrated scale of the BodPod (Life Measurement) while patients were only wearing underwear and after an overnight fast. BMI was calculated by dividing bodyweight with height squared (kg/m^2). While the subjects are in a fasting state in the morning, their hip, waist and thigh circumference was measured. Hip circumference (cm) was measured at the largest circumference between waist and thighs and waist circumference was measured at the smallest circumference between the ribcage and the iliac crest. Thigh circumference (cm) was measured at the largest circumference of the upper right leg.

Body composition measurements were done by BodPod (Version 4.2.0, Life Measurements, inc). This technique is one of the least invasive techniques to test body composition and it uses air displacement to measure body volume and to evaluate the changes in FM and FFM at baseline, after 8 weeks (after LCD) and after 26 weeks.

6.5 Glucose intolerance

An OGTT will be performed on every subject. At the start of the OGTT, the participant must be in a fasted state. During this test, each participant will take an oral load of 82.5g dextrose-monohydrate (Avebe, the Netherlands) dissolved in 300ml water. After this glucose intake, the participant is not allowed to drink or eat anything. Blood samples will

be obtained during the OGTT for the measurement of glucose at baseline and 120 minutes after the glucose intake in order to identify possible new cases of T2DM and to evaluate the possible changes in plasma glucose concentration during and after dietary intervention. An OGTT will be performed at baseline and after 26 weeks.

6.6 Physical fitness

Physical fitness will be investigated with the use of an incremental ergometer test which measures respiratory gases and this test will be performed while the participants are in a fasting state The outcome measurement of this test is maximal oxygen consumption (VO2max, reflects aerobic physical fitness). This test is submaximal up to an estimated 85% of VO2max and the test will be performed at baseline (before the weight reduction period) and after 26 weeks.

In order to measure habitual physical activity of the participants, the validated Dutch translation of the Baecke questionnaire was used at baseline and after 26 weeks. The questionnaire uses work, sport and leisure time as the tree indices for physical activity. Next to the Baecke questionnaire, the participants received an accelerometer to evaluate their amount and type of physical activity. These accelerometers (Actigraph) will be worn for seven consecutive days during day and night at baseline and after 26 weeks.

6.7 Statistics

Data are presented in means ± Standard deviations (SDs). Not normally distributed parameters were log transformed. Differences in baseline parameters between both exercise groups were evaluated using independent sample t-tests. Factorial ANOVA was used to test the change over time (baseline and after 26 weeks) between both physical activity groups for accelerometers, baecke questionnaires and VO2max. Repeated measures ANOVA was used to investigate differences between baseline (clinical investigation day 1, CID1), 8 weeks (CID2) and 26 weeks (CID3). Baseline values were used as covariate when they were significantly different between both groups. Pearson and spearman (when parameters were not normally distributed) correlations were used to determine relationships between different parameters. Differences were considered significant if $P < 0.05$. All analyses were performed with SPSS version 21 for windows.

7 Results

7.1 Baseline characteristics of the participants

MI and HI physical activity groups were compared for baseline characteristics of the participants (Table 2). Age, height, BMI, bodyweight (BW), FM, FFM, body fat (BF), waist to hip ratio (W:H ratio), VO2max, baecke questionnaires (BAQ), accelerometers and blood glucose concentration were measured at baseline. A significant difference between both groups is present for height, BW, FFM and the work index of the baecke questionnaires (BAQ: work). Baseline characteristics for the whole group are also calculated (Table 2).

Table 2. Baseline characteristics of participants.

	Moderate intensity (n=18)	High intensity (n=15)	All subjects (n=33)
Age, y	53.0 ± 10,3	59.0 ± 9,1	55.8 ± 10.0
Height, m	1,77 ± 0,08*	1,68 ± 0,08*	1.73 ± 0.10
BMI, kg/m²	33,26 ± 4,07	31,98 ± 5,86	32.68 ± 4.92
BW, kg	104,66 ± 16,68*	90,06 ± 18,04*	98.02 ± 18.57
FM, kg	42,89 ± 10,73	38,40 ±12,24	40.85 ± 11.48
FFM, kg	61,77 ± 10,15*	51,65 ± 12,19*	57.17 ± 12.08
BF, %	40,73 ± 6,33	42,48 ± 8,71	
W:H ratio	,97 ± 0,11	,96 ± 0,18	0.97 ± 0.14
VO2max (ml/kg BW/min)	24,28±4,16	22,07±4,44	23,28 ± 4,36
BAQ: Work	2,42 ± 0,89*	3,03 ± 0,76*	2.70 ± 0.88
BAQ: Sport	2,90 ± 0,96	2,56 ± 1,11	2.74 ± 1.03
BAQ: Leisure	2,75 ± 0,73	3,15 ± 0,50	2.93 ± 0.66
BAQ: Total	8,06 ± 1,42	8,74 ± 1,78	8.37 ± 1.60
Activity intensity:			
Sedentary, %	81,66 ± 4,15	79,77 ± 3,85	80.87 ± 4.07
Light, %	9,47 ± 2,98	10,62 ± 2,96	9.95 ± 2.98
Lifestyle, %	5,21 ± 1,20	5,72 ± 1,91	5.42 ± 1.53
Moderate, %	3,57 ± 1,33	3,82 ± 2,40	3.67 ± 1.82
Vigorous, %	,10 ± 0,16	,05 ± 0,05	0.08 ± 0.12
Very vigorous, %	,00 ± 0,00	,01 ± 0,02	0.003 ± 0.01
Counts/day	890255,96 ± 253576,91	1054197,60 ± 359107,98	966371,72 ± 312392,09
Glucose (0h), mmol/L	5,88 ± 0,54	6,14 ± 0,80	6.00 ± 0.66
Glucose (2h), mmol/L	6,46 ± 1,96	7,14 ± 1,47	6.76 ± 1.77

A significant difference between the moderate and the high intensity physical activity group is present for height, BW, FFM and the work index, which is obtained and calculated via the baecke questionnaires. *Difference between moderate en high intensity physical activity groups, $P < 0.05$. BMI, body mass index; BW, bodyweight; FM, fat mass; FFM, fat free mass; BF, body fat; W:H ratio, waist to hip ratio; VO2max, maximal oxygen consumption (corrected for BW); BAQ, Baecke Activity Questionnaire.

7.2 Difference between moderate and high intensity physical activity

In order to investigate if the intervention was successful, different physical activity parameters were compared to investigate whether there is an actual difference in physical activity intensity between both groups. Participants were asked to wear accelerometers for seven consecutive days at baseline and after 26 weeks. Outcome values of the accelerometers are percentage sedentary, light, lifestyle, moderate, vigorous and very vigorous physical activity. In addition, counts per day were also used to evaluate physical activity. However, there were no significant differences between the MI and the HI physical activity group (Table 3).

Table 3. Accelerometers (actigraph) are used to assess the physical activity of the participants.

Activity intensity	Moderate intensity (n=13)	High intensity (n=9)
Change in sedentary, %	-8.48 ± 6.69	-7.65 ± 9.07
Change in light, %	7.31 ± 5.47	7.59 ± 7.29
Change in lifestyle, %	1.20 ± 1.82	0.29 ± 2.34
Change in moderate, %	-0.08 ± 1.49	-0.61 ± 1.80
Change in vigorous, %	0.05 ± 0.14	0.38 ± 1.01
Change in very vigorous, %	0.00 ± 0.01	0.00 ± 0.00
Change in counts/day	108823,44 ± 80464,77	25199,84 ± 89002,32

Accelerometers were used to evaluate physical activity of the participants at baseline and after 26 weeks. Outcome values used after calculation are counts per day and percentage sedentary, light, lifestyle, moderate, vigorous and very vigorous physical activity. Percentage sedentary and moderate physical activity decreased after a LCD in combination with a moderate protein diet, but the other values increased. However, there was no significant difference between the MI and the HI physical activity group for all the outcome values.

Together with the accelerometers, a baecke questionnaire was used to evaluate the physical activity of the participants. The outcome values of this questionnaire are work index, sport index, leisure index and baecke total. The work index of the participants slightly decreased after a LCD in combination with a moderate protein diet in both physical activity groups, but the other outcome values increased (Table 4). However, these results were not significant and no significant differences between the MI (n=18) and HI (n=15) physical activity group could be detected for any of the outcome values (Table 4).

Table 4. Outcome values of the baecke questionnaire.

	Moderate intensity (n=18)	High intensity (n=15)
BAQ: change in work	-0.09 ± 0.85	-0.18 ± 0.82
BAQ: change in sport	0.46 ± 0.61	0.55 ± 0.72
BAQ: change in leisure	0.60 ± 0.83	0.37 ± 0.80
BAQ: change in total	0.97 ± 1.46	0.74 ± 1.03

Baecke questionnaires are used to assess the physical activity of the participants. Outcome values of this questionnaire are work index, sport index, leisure index and baecke total. However, there was no significant difference between the MI and the HI group for all the outcome values. MI, moderate intensity physical activity (n=16); HI, high intensity physical activity (n=15)

Physical fitness of the participants was evaluated by measuring VO2max of the participants with the use of an incremental ergometer test. VO2max was measured at baseline and after 26 weeks and corrected for bodyweight. The VO2max of the participants did increase after a LCD in combination with a moderate protein diet (Figure 2). However, there was no significant difference between the MI (n=16; -0.39±1,13) and the HI (n=15; -0,10±1,05) physical activity group (Figure 2).

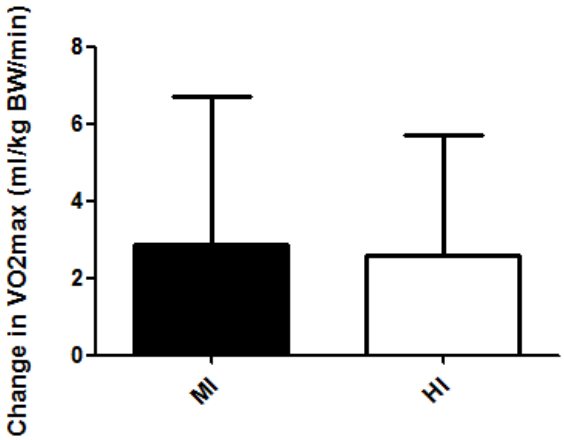

Figure 2. Change in VO2max was measured with an incremental ergometer test and corrected for bodyweight. Change in VO2max was not significantly different between the MI and HI physical activity group. However, VO2max increased after a LCD followed by a moderate protein diet. VO2max was corrected for bodyweight. BW, bodyweight; MI, moderate intensity physical activity (n=16); HI, high intensity physical activity (n=15)

Both physical activity groups did not significantly differ from each other when accelerometers, baecke questionnaires and VO2max are taken into account. Therefore, further analyses will be performed on the total group of subjects. The effect of overall physical activity will be investigated instead of comparing MI physical activity with HI physical activity.

7.3 The effect of physical activity and moderate protein diet on bodyweight and body composition

Bodyweight was measured with a calibrated scale (Life Measurement) of the BodPod at baseline (CID1; 98,02±18,57kg), after eight weeks (CID2; 86,24±15,65kg) and after 26 weeks (CID3; 87,00±16,22kg). Bodyweight (n=33) significantly decreased between CID1 and CID2 (p=0,000) and between CID1 and CID3 (p=0,000). However, bodyweight did not significantly change between CID2 and CID3 (Figure 3).

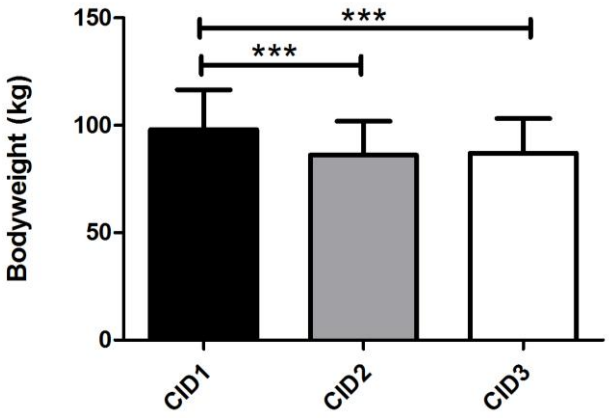

Figure 3. Bodyweight was measured with a calibrated scale of the BodPod. Bodyweight significantly decreased between CID1 and CID2 (after the low calorie diet, p=0,000) and between CID1 and CID3 (after low calorie diet and moderate protein diet, p=0,000). However, there was no significant decrease between CID2 and CID3. n=33. CID, clinical investigation day.

Bodyweight and height were measured and used to calculate BMI at CID1 (32,68±0,86kg/m²), CID2 (28,80±0,77kg/m²) and CID3 (29,03±0,79kg/m²). BMI (n=33) significantly decreased between CID1 and CID2 (p=0,000) and between CID1 and CID3 (p=0,000). However, there was no significant difference between CID2 and CID3 (Figure 4).

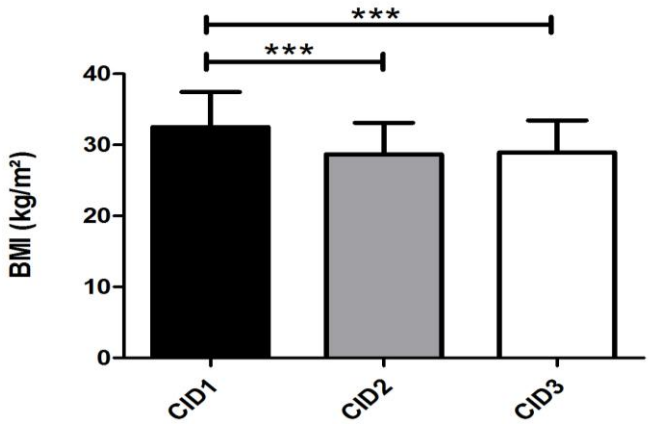

Figure 4. BMI was calculated after measuring height and bodyweight. A significant decrease of BMI is present between CID1 and CID2 (p=0,000) and between CID1 and CID3 (p=0,000). However, there was no significant decrease of BMI between CID2 and CID3. n=33. BMI, body mass index; CID, clinical investigation day.

The BodPod was used to measure the amount of FM of the participants at CID1 (40,85±11,48kg), CID2 (30,86±10,41kg) and CID3 (30,56±10,69kg). FM (n=33) significantly decreased between CID1 and CID2 (p=0,000), and between CID1 and CID3 (p=0,000). However, there was no significant difference between CID2 and CID3 (Figure 5).

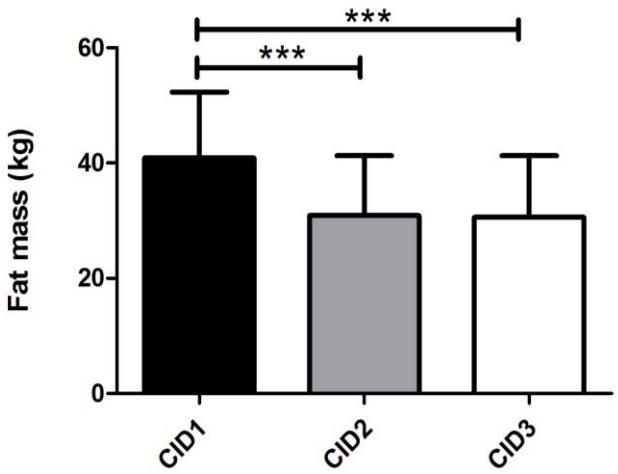

Figure 5. Fat mass was measured with the use of a BodPod. Fat mass significantly decreased between CID1 and CID2 (p=0,000), and between CID1 and CID3 (p=0,000). However, there was no significant decrease of fat mass between CID2 and CID3. n=33. CID, clinical investigation day.

In addition to FM, changes in FFM were also evaluated. FFM was, like the other body composition parameters, measured at CID1 (57,17±2,10kg), CID2 (55,39±1,93kg) and CID3 (56,44±2,02kg). FFM (n=33) significantly decreased between CID1 and CID2 (p=0,000), and between CID1 and CID3 (p=0,028). However, FFM significantly increased between CID2 and CID3 (p=0,000) (Figure 6).

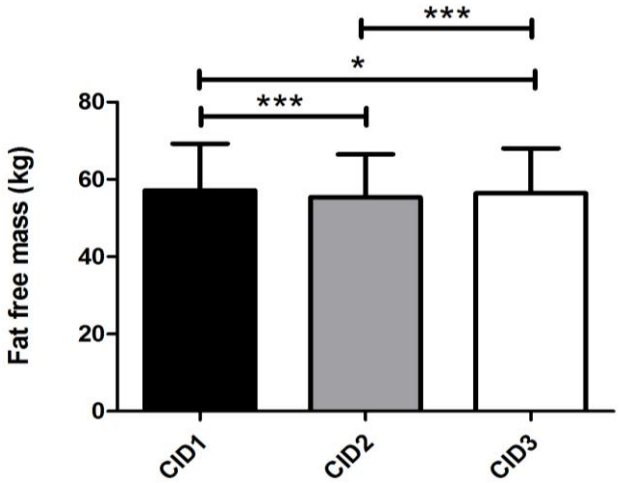

Figure 6. Fat free mass was measured with the use of a BodPod. Fat free mass significantly decreased between CID1 and CID2 (p=0,000) and between CID1 and CID3 (p=0.028). However, fat free mass significantly increased again between CID2 and CID3 (p=0,000). n=33. CID, clinical investigation day.

The BodPod was used to measure body fat percentage at CID1 (41,52±1,30%), CID2 (30,86±1,81%) and CID3 (34,82±1,52%). Body fat percentage (n=33) significantly decreased between CID1 and CID2 (p=0,000), and between CID1 and CID3 (p=0,000). However, body fat percentage significantly increased between CID2 and CID3 (p=0.005, Figure 7).

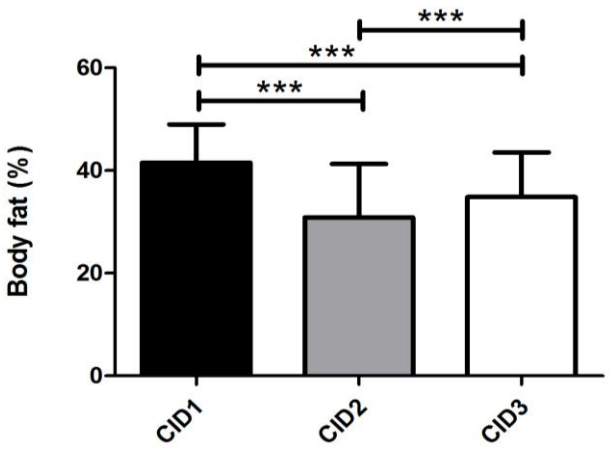

Figure 7. Body fat percentage was measured with the use of a BodPod. Body fat percentage significantly decreased between CID1 and CID2 (p=0,000), and between CID1 and CID3 (p=0,000). However, body fat percentage increased again between CID2 and CID3 (p=0,005). n=33. CID, clinical investigation day.

Anthropometric measurements for waist to hip ratio were performed at CID1 (0,97±0.03), CID2 (0,92±0.02) and CID3 (0,93±0.02). Waist to hip ratio (n=33) significantly decreased between CID1 and CID2 (p=0,011). However, there was no significant difference between CID1 and CID3, or between CID2 and CID3 (Figure 8).

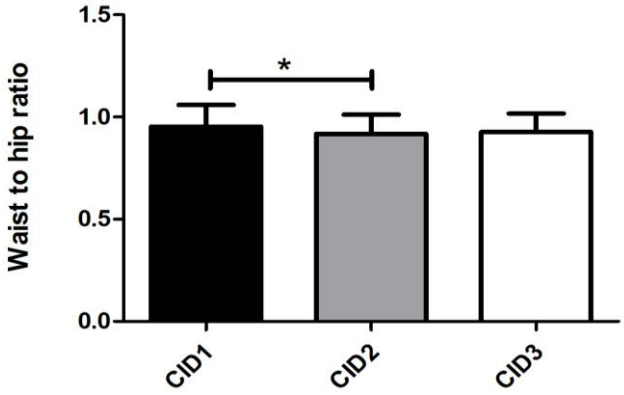

Figure 8. Waist to hip ratio was calculated based on the anthropometric measurements. Waist to hip ratio significantly decreased between CID1 and CID2 (p=0,011), but there was no significant difference between CID1 and CID3, or between CID2 and CID3. n=33. CID, clinical investigation day.

7.4 The effect of physical activity and moderate protein diet on glucose metabolism

In order to measure glucose tolerance, all participants performed an OGTT at CID1 (A.6,00±0,12mmol/L; B.6,76±0,31mmol/L) and CID3 (A.5,53±0,081mmol/L; B.5,64±0,27mmol/L). Fasted blood glucose concentration (n=32) significantly decreased between CID1 and CID3 (p=0,000, Figure 9A). However, there was no significant difference between CID1 and CID3 when the blood glucose concentration two hours after an OGTT (n=32) was evaluated (Figure 9B).

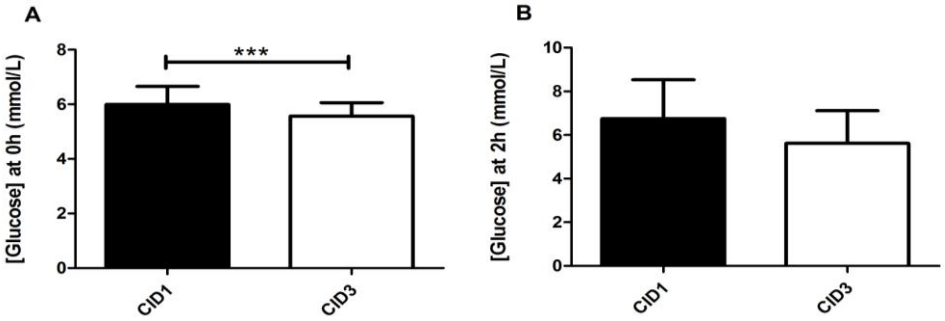

Figure 9. Glucose concentration was measured by performing an oral glucose tolerance test (OGTT). Fasted blood glucose concentration significantly decreased between CID1 and CID3 (A; p=0,000). However, blood glucose concentration two hours after an OGTT did not significantly differ between CID1 and CID3. n=32. CID, clinical investigation day; [glucose], glucose concentration.

7.5 Association between body composition and blood glucose concentration

Correlation coefficients were computed to evaluate possible associations between the change of different parameters of body composition and blood glucose concentration. However, no significant correlations were found between body composition and blood glucose concentration, except for the correlation between fasted blood glucose concentration and glucose concentration two hours after an OGTT, which is obvious (Table 5).

Table 5. Correlation between change of different parameters of body composition and change of blood glucose concentration.

	BMI (n=33)	BW (n=33)	FM (n=33)	FFM (n=33)	BF% (n=33)	W:H ratio (n=33)	Glucose (0h, n=32)	Glucose (2h, n=32)
Glucose (0h, n=32)	-,064	-.090	-.041	-.178	.089	.018	1	,415*
Glucose (2h, n=32)	.207	-.077	-.122	.149	-.277	-.235	,415*	1

Correlation coefficients were computed to determine the association between change in body composition and change in blood glucose concentration. However, no significant correlations were found. Parameters used are change between CID1 and CID3. Values are correlation coefficients. BMI, body mass index; BW, bodyweight; FM, fat mass; FFM, fat free mass; BF%, body fat percentage; W:H ratio, waist to hip ratio; Glucose (0h), fasted blood glucose concentration; Glucose (2h), blood glucose concentration 2h after an OGTT; CID, clinical investigation day.

7.6 Association between physical activity and body composition

Correlation coefficients were computed to evaluate possible associations between the change in VO2max, change in outcome values of the baecke questionnaires, change in outcome values of the accelerometers and change in body composition. A significant positive correlation was found between FFM and sport index of the baecke questionnaire (r=0,360, p=0,039). In addition, significant negative correlations were found between VO2max and bodyweight (r=-0,384, p=0,027), VO2max and body fat percentage (r=-0,560, p=0,001), VO2max and FM (r=-0,417, p=0,016), and VO2max and BMI (r=-0,450, p=0,009).

7.7 Association between the change of different parameters of physical activity and physical fitness.

Correlation coefficients were computed to evaluate possible associations between the change in VO2max, change in outcome values of the baecke questionnaires and change in outcome values of the accelerometers. However, there were no significant correlations found between change in VO2max and the change in outcome values of the baecke questionnaires or between the change in VO2max and the change in outcome values of the accelerometers. In addition, change in outcome values of baecke questionnaires and accelerometers are not significantly correlated (Table 6).

Table 6. Association between the change of different parameters of physical activity and physical fitness.

	VO2max (n=31)	BAQ: work (n=33)	BAQ: sport (n=33)	BAQ: leisure (n=33)	BAQ: total (n=33)	%Sedentary (n=22)	%Light (n=22)	%Lifestyle (n=22)	%Moderate (n=22)	%Vigorous (n=22)	%Very vigorous (n=22)	Counts /day
VO2max (n=31)	1	.292	.203	-.293	.127	.113	-.108	-.176	.074	.082	-.037	,172
BAQ: work (n=33)	.292	1				-.049	-.024	.063	.001	-.147	.325	-,304
BAQ: sport (n=33)	.203		1			.100	-.097	-.095	-.100	.296	-.105	-,088
BAQ: leisure (n=33)	-.293			1		-.208	.131	.029	,467*	-.057	-.293	,335
BAQ: total (n=33)	.127				1	-.113	.022	.029	.275	.106	-.186	,045
%Sedentary (n=22)	.113	-.049	.100	-.208	-.113	1						-,120
%Light (n=22)	-.108	-.024	-.097	.131	.022		1					-,042
%Lifestyle (n=22)	-.176	.063	-.095	.029	.029			1				,115
%Moderate (n=22)	.074	.001	-.100	,467*	.275				1			,546*
%Vigorous (n=22)	.082	-.147	.296	-.057	.106					1		,297
%Very vigorous (n=22)	-.037	.325	-.105	-.293	-.186						1	,064
Counts/day (n=22)	,172	-,304	-,088	,335	,045	-,120	-,042	,115	,546*	,297	,064	1

Correlation coefficients were computed to determine the possible associations between change of VO2max, change in outcome values of the baecke questionnaires and change in outcome values of the accelerometers. There were no significant correlations between the different parameters. Values are correlation coefficients. VO2max, maximal oxygen consumption (corrected for bodyweight); BAQ, Baecke Activity Questionnaire.

8 Discussion

The main goal of this research project was to investigate if a different physical activity intensity could lead to better prevention of T2DM through improvement of the body composition, glucose tolerance and physical fitness. Forty three subjects were enrolled in this study from which ten dropped out. Different effects of MI and HI physical activity on body composition, glucose tolerance and physical fitness were tested in the remaining thirty three subjects.

First of all, results of the accelerometers, baecke questionnaires and the VO2max test were used to investigate whether the intervention was successful. The results of the accelerometers and the baecke questionnaires were evaluated and it seems that the HI group increased its percentage vigorous activity more than the MI group and the MI group decreased their change in percentage moderate activity less compared to the HI group. However, these results were not significant and therefore both groups did not significantly differ in exercise intensity. This means that both groups exercised more or less at the same intensity. However, it must be taken into account that only twenty two subjects could be used for the accelerometers due to loss of the accelerometer or not recording by the accelerometer.

In addition, physical fitness was evaluated and the difference between baseline and after 26 weeks of intervention was calculated. There was no significant difference between the MI and the HI physical activity group. However, a higher increase of the physical fitness was expected in the HI group compared to the MI group, because different studies have showed that HI physical activity is more effective for the improvement of VO2max. In a study of T. Matsuo et al., the effect of exercise intensity on VO2max was investigated in overweight male subjects. All subjects underwent a cycling exercise intervention in which they exercised three times a week for eight weeks. They compared the effect of MI and HI on physical fitness and their results showed an improvement of the VO2max in both groups, but a higher increase was present in the HI physical activity group (29).

All previous results show that there is no significant difference between the MI and the HI physical activity group, when exercise intensity is evaluated. This means that the intervention was not successful and that in fact both groups exercised at about the same intensity. Therefore, it was decided to take both groups together for further analysis and to look at the effect of physical activity in combination with moderate protein diet instead of looking at the different effects of exercise intensity.

A BodPod was used in order to investigate the effect of physical activity and moderate protein diet on body composition. In addition, BMI was calculated based on bodyweight and height measurements. BodPod measurements and BMI calculations were performed

before (CID1) and after a LCD (CID2) and during a moderate protein diet (CID3). Bodyweight, BMI, FM, body fat percentage and FFM were evaluated and all parameters significantly decreased between CID1 and CID2, but also between CID1 and CID3. However, there was no significant difference in bodyweight, BMI and FM between CID2 and CID3. FFM and body fat percentage did increase again between CID2 and CID3, the increase in FFM is probably caused by an increase in physical activity during the intervention. The results were expected, except for the increase of body fat percentage between CID1 and CID2, because the goal of the intervention was to lose weight during the LCD and to maintain this weight during the intervention. In addition, the aim was to have a greater loss of FM and a preservation of the FFM. This goal is not quite achieved because body fat percentage significantly increased again after LCD and it was expected that body fat percentage would be maintained or even decrease further after the LCD.

In a study of D. K. Layman et al., overweight and obese women underwent a diet with a higher amount of proteins in combination with regular exercise sessions. Results showed that a diet higher in proteins led to more weight loss compared to a diet low in proteins. In addition, protein diet in combination with physical activity led to a higher decrease of the FM and a better preservation of the FFM (14). Another study, in which overweight adults received dietary guidelines in combination with supervised exercise sessions, found that this intervention compared to no exercise led to a greater decrease of the bodyweight (30). A possible explanation for these contrasting findings is that lifestyle intervention was not strictly controlled in our study, which caused an increase of the bodyweight and FM after CID2 in some subjects.

Anthropometric measurements were also performed at CID1 , CID2 and CID3, and they were used to calculate waist to hip ratio. Waist to hip ratio did only significantly differ between CID1 and CID2. However, there was no longer a difference in waist to hip ratio when the intervention was also taken into account (between CID1 and CID3). However, a further decrease or maintenance of the waist to hip ratio was expected. The research group of L. Burke et al. investigated changes in waist to hip ratio in older, overweight and obese adults. Participants received dietary guidelines and they were motivated to move more. Results of this study showed a significant decrease of the waist to hip ratio after intervention (31).

Next, glucose tolerance was evaluated in the pre-diabetic participants, in order to investigate if glucose tolerance improves after intervention. Fasted blood glucose concentration did significantly decrease between CID1 and CID3. Blood glucose concentration two hours after the OGTT did not significantly differ between CID1 and CID3. However, a decrease of blood glucose concentration two hours after an OGTT was expected. A study, in which normal weight young adults performed a single exercise bout

on a cycle ergometer, found that one single bout of physical activity was enough to lower blood glucose concentration (26). Another study of R. Ross et al., investigated the effects of exercise intensity on glucose tolerance in obese adults, in which LI physical activity was compared with HI physical activity. Participants exercised on a treadmill five times a week for 24 weeks. However, a reduction of the two hour blood glucose level was only present after HI physical activity (25). This could explain why blood glucose concentration two hours after an OGTT did not differ between CID1 and CID3. The exercise intensity of our participants was probably too low in order to see an effect.

Finally, Correlation coefficients were computed to evaluate possible associations between the change in VO2max, change in outcome values of the baecke questionnaires, change in outcome values of the accelerometers and change in body composition. A significant positive correlation was found between FFM and sport index of the baecke questionnaire. This means that the FFM increases when the sport index increase, which is expected because more exercise leads to the increase of the FFM (14, 15).

In addition, significant negative correlations were found between VO2max and bodyweight, VO2max and body fat percentage, VO2max and FM, and VO2max and BMI. This means that VO2max increases when bodyweight, FM, BMI and body fat percentage decrease, which is also expected because literature describes that physical activity improves when subjects lose weight. A study of J. M. Jakicic et al. investigated the effect of lifestyle intervention on physical fitness in overweight or obese people with T2DM. They compared lifestyle intervention to diabetes support and education and their results showed that the group who received lifestyle intervention lost more weight and had a better improvement of their physical fitness (32). Another study, in which young adults with a normal weight were used to investigate possible associations between body composition and physical fitness, found an inverse association between body composition and fitness score. In addition, BMI was inverse correlated with physical fitness score. However, they used the modified Harvard Step test instead of an incremental ergometer test to assess physical fitness (33).

In conclusion, the results found in this study did not show a difference in exercise intensity, therefore both intensity groups were taken together. This lack of a difference between both groups could be prevented when exercise was supervised, but because this study is part of the PREVIEW study, in which several hundred participants take part, supervised exercise sessions could not be organised. However, literature in which supervised exercise sessions were used, did found different effects of different exercise intensities. A study of V. Mougios et al., performed in normal to overweight women, used hydrostatic weighing to measure body composition in participants who performed a supervised exercise session four times a week for three months. They found a decrease

of the FFM in most of the subjects of the LI group and an increase of the FFM in the high intensity group (34). Another study of M.A. Grediagin et al. also investigated the effect of exercise intensity in body composition in moderately overfat women (BMI between 23.8±2.3kg/m² and 26.2±1.4kg/m²). Subjects exercised four times a week for twelve weeks on a treadmill. Results of this study showed a higher increase of the FFM in the HI group compared to the LI physical activity group, but in this study both groups lost an equal amount of FM. Therefore, subjects of the LI group lost more bodyweight compared to subjects in the HI physical activity group (35). In addition, a study of W. Guo et al. in normal to overweight women, showed that an increased amount of HI physical activity was associated with a lower waist to hip ratio, but also a lower BMI and body fat percentage (36).

9 Conclusion and future prospective

Obesity is a worldwide problem that is rising fast. It probably is the cause of different metabolic diseases, like T2DM. Once T2DM is present, the disease is not reversible. Therefore, the prevention of T2DM is very important. In this study, lifestyle intervention is used in order to prevent T2DM in overweight or obese pre-diabetic adults. Changes in body composition, glucose tolerance and physical fitness were investigated within two intervention groups, moderate protein diet in combination with MI or HI physical activity.

First of all accelerometers, baecke questionnaires and VO2max tests were evaluated in order to investigate if the intervention was successful. However, no significant differences were found between the MI and HI physical activity group, which means that both groups exercised at almost the same intensity. Therefore, it was decided to take both groups together for further analysis and investigate the effect of overall physical activity on body composition and glucose tolerance.

All body composition parameters significantly decreased between CID1 and CID2, and between CID1 and CID3. FFM and body fat percentage significantly increased again between CID2 and CID3. Waist to hip ratio did only significantly decreased between CID1 and CID2. In addition, an OGTT was performed in order to evaluate changes in glucose tolerance in these pre-diabetic subjects. Fasted blood glucose concentrations significantly decreased between CID1 and CID3. However, there was no significant difference between CID1 and CID3 for blood glucose concentration two hours after an OGTT. Finally, correlation coefficients were computed and a significant positive correlation was found between FFM and the sport index obtained via the baecke questionnaire. In addition, negative correlations were found between VO2max and bodyweight, VO2max and FM, VO2max and BMI, and VO2max and body fat percentage.

Based on these results, it can be concluded that physical activity in combination with a moderate protein diet, improves body composition by decreasing bodyweight, BMI, FM, body fat percentage and waist to hip ratio. Intervention was also successful for the preservation of FFM and the improvement of the fasted blood glucose concentration. However, a drawback of this study is that the physical activity intensity was not strictly controlled which led to the fact that there was no significant difference in exercise intensity between both groups. Therefore, it is really important to investigate the effect of exercise intensity on these parameters in a follow-up study in which physical activity is highly controlled and supervised. The effectiveness of different exercise intensities for the prevention of T2DM can be investigated and possible adjustments in lifestyle intervention can be used in the clinic to prevent T2DM in pre-diabetic overweight or obese adults.

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11 Supplementary information

11.1 Inclusion and exclusion criteria

11.1.1 Inclusion criteria

1) Age must be between 25 and 70 years;
2) Overweight or obesity status (BMI>25 kg/m^2)
3) Pre-diabetes: the criteria form the WHO/IDF (International Diabetes Foundation) for assessing pre-diabetes will be used as the formal inclusion criteria, i.e. having:
    - Impaired Fasting Glucose (IFG): Fasting venous plasma glucose concentration 5.6-6.9 mmol/l or
    - Impaired Glucose Tolerance (IGT): Venous plasma glucose concentration of 7.8-11.0 mmol/l at two hours after oral administration of 82.5g dextrose-monohydrate (oral glucose tolerance test, OGTT), with fasting plasma glucose less than 7.0 mmol/l.
4) An informed consent is required;
5) Smoking is allowed, provided subjects have not recently (within one month) changed their smoking habits. However, smoking status is monitored throughout the study and used as confounding variable;
6) Motivation and willingness to be randomized to any of the groups and to do his/hers best to follow the given protocol;
7) Able to participate at clinical investigation days (CID's) during normal working hours;

11.1.2 Exclusion criteria

Medical conditions as known by the subjects:

1) Diabetes mellitus (other than gestational diabetes mellitus);
2) Significant cardiovascular disease including current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease;
3) Systolic blood pressure above 160 mmHg and/or diastolic blood pressure above 100 mmHg whether on or off treatment for hypertension. If being treated, no change in drug treatment within last 3 months;
4) Advanced chronic renal impairment;
5) Significant liver disease e.g. cirrhosis (fatty liver disease allowed);
6) Malignancy which is currently active or in remission for less than five years after last treatment (local basal and squamous cell skin cancer allowed);

7) Active inflammatory bowel disease, celiac disease, chronic pancreatitis or other disorder potentially causing malabsorption;
8) Previous bariatric surgery;
9) Chronic respiratory, neurological, musculoskeletal or other disorders where, in the judgment of the investigator, participants would have unacceptable risk or difficulty in complying with the protocol (e.g. physical activity program);
10) A recent surgical procedure until after full convalescence (investigators judgment);
11) Transmissible blood-borne diseases e.g. hepatitis B, HIV;
12) Psychiatric illness (e.g. major depression, bipolar disorder).

Medication:

13) Use currently or within the previous 3 months of prescription medication that has the potential of affecting bodyweight or glucose metabolism such as glucocorticoids (but excluding inhaled and topical steroids; bronchodilators are allowed), psychoactive medication, epileptic medication, or weight loss medications (either prescription, over the counter or herbal). Low dose antidepressants are allowed if they, in the judgment of the investigator, do not affect weight or participation to the study protocol. Levothyroxine for treatment of hypothyroidism is allowed if the participant has been on a stable dose for at least 3 months.

Personal/Other:

14) Engagement in competitive sports;
15) Self-reported weight change of >5 % (increase or decrease) within 2 months prior to screening;
16) Special diets (e.g. vegan, Atkins) within 2 months prior to study start. A lacto-vegetarian diet is allowed;
17) Severe food intolerance expected to interfere with the study;
18) Regularly drinking > 21 alcoholic units/week (men), or > 14 alcoholic units/week (women);
19) Use of drugs of abuse within the previous 12 months;
20) Blood donation or transfusion within the past 1 month before baseline or CID's;
21) Self-reported eating disorders;
22) Pregnancy or lactation, including plans to become pregnant within the next 36 months.
23) No access to either phone or Internet (this is necessary when being contacted by the instructor's during the maintenance phase);

24) Inadequate understanding of national language;
25) Psychological or behavioral problems which, in the judgment of the investigator, would lead to difficulty in complying with the protocol.

Laboratory screening:

If all of the above criteria are satisfied, the participant is eligible for a glucose tolerance test (blood at 0 and 120 min), and blood glucose concentrations are analyzed immediately (analyzer, Radiometer, Copenhagen).

ONLY IF the glucose tolerance test meets the entry criteria for the study, the remaining samples are sent to the local laboratory for a safety check, with the following exclusion criteria:

26) Haemoglobin concentration below local laboratory reference values (i.e. anaemia).
27) Creatinine >1.5 times Upper Limit of Normal (local laboratory reference values).

Auteursrechtelijke overeenkomst

Ik/wij verlenen het wereldwijde auteursrecht voor de ingediende eindverhandeling:
Prevention of Type II diabetes mellitus through lifestyle intervention in predisposed obese adults

Richting: **master in de biomedische wetenschappen-klinische moleculaire wetenschappen**
Jaar: **2015**

in alle mogelijke mediaformaten, - bestaande en in de toekomst te ontwikkelen - , aan de Universiteit Hasselt.

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Datum: **9/06/2015**