Original Article

High intensity interval training is associated with greater impact on physical fitness, insulin sensitivity and muscle mitochondrial content in males with overweight/obesity, as opposed to continuous endurance training: a randomized controlled trial

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Abstract

Objectives: To evaluate the effect of high intensity training (HIT) on physical fitness, basal respiratory exchange ratio (bRER), insulin sensitivity and muscle histology in overweight/obese men compared to continuous aerobic training (CAT). **Material and methods:** 16 male participants with overweight/obesity (age: 42-57 years, body mass index: 28-36 kg/m^2) were randomized to HIT (n=8) or CAT (n=8) for 10 weeks, twice a week. HIT was composed of 10 minutes high intensity, 10 minutes continuous aerobic, 10 minutes high intensity exercises. CAT was composed of three times 10 minutes continuous exercising. Changes in anthropometry, physical and metabolic fitness were evaluated. Muscle histology (mitochondria and lipid content) was evaluated by transmission electron microscopy (TEM). **Results:** HIT showed a significant increase for peak VO2 (P=0.01), for insulin sensitivity (AUC glucose (P<0,001), AUC insulin (P<0,001), OGTT composite score (P=0.007)) and a significant decrease of bRER (P<0.001) compared to CAT. Muscle mitochondrial content was significantly increased after HIT at the subsarcolemmal (P=0.004 number and P=0.001 surface) as well as the intermyofibrillar site (P<0.001 number and P=0.001 surface). **Conclusion:** High intensity training elicits stronger beneficial effects on physical fitness, basal RER, insulin sensitivity, and muscle mitochondrial content, as compared to continuous aerobic training.

Keywords: Insulin Sensitivity, High Intensity Interval Training, Overweight/Obesity, Mitochondria

Introduction

Regular physical exercise leads to improvements in glycaemia control[1,2], body composition[3-5], cardiovascular risk factors[4,5] and exercise capacity[3,5] in patients with Type 2 Diabetes Mellitus or Insulin Resistance[1,3,6]. Exercise training has beneficial effects on insulin sensitivity and glycaemia control through the stimulation of mitochondrial function[7-9]. Though training volume has been suggested to be a primary determinant of exercise-induced increase in mitochondrial content in humans[10], hypothesis now rise that exercise training at higher intensities (HIT) may provoke even greater mitochondrial adaptations compared to lower exercise intensities in healthy humans[7,11-13].

HIT triggers a pathway leading to increased mitochondrial protein synthesis rates and mitochondrial respiration and thus greater increase in mitochondrial content compared to lower exercise intensities[7,14]. Besides, HIT causes increased use of carbohydrates for
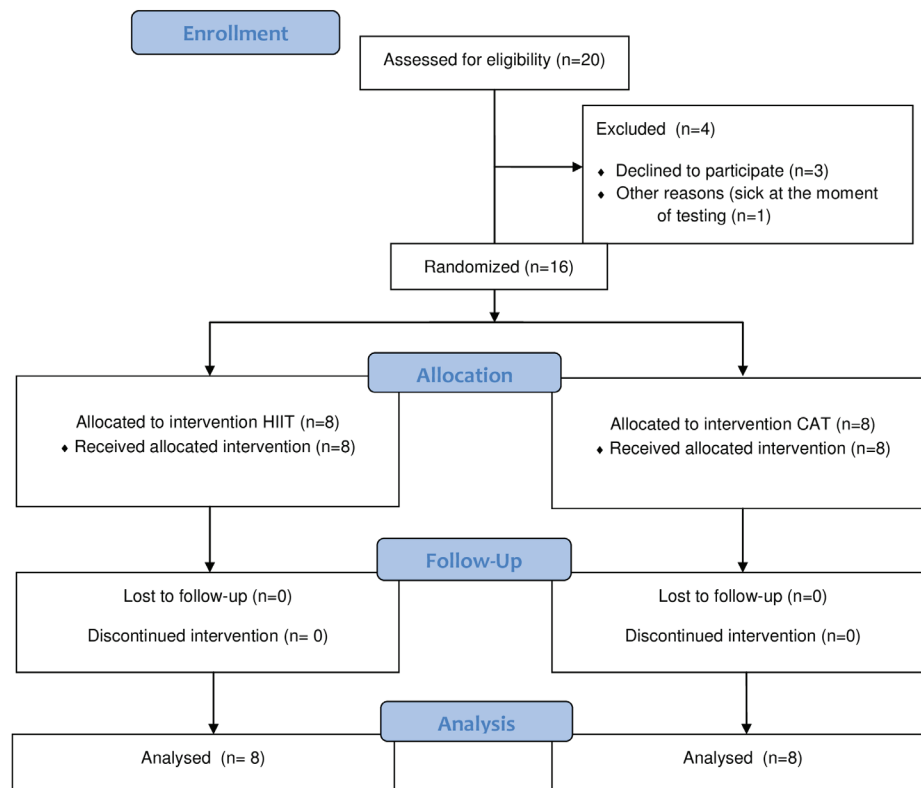
Figure 1. Patient Flow diagram. HIT: high intensity training; CAT: continuous aerobic training

fuel compared to low intensity exercises where lipids are used dominantly[7]. The few studies that have compared continuous endurance training and HIT protocols in healthy humans have yielded inconsistent findings, with higher exercise intensities eliciting greater mitochondrial adaptations in some comparisons[7,9,15] but not in others[15,16].

Especially in type 2 diabetes mellitus, HIT has specific 'diabetes-associated' beneficial effects as improved: (i) cardio metabolic health, (ii) insulin sensitivity and (iii) chronic glycaemia control (as expressed in HbA1c) when compared to no exercise or to continuous endurance training[17]. Though, research examining the effects of HIT in patients at risk for type 2 diabetes mellitus show inconsistent results, with increased insulin sensitivity[18-21], no effects of HIT[22-25], or even larger beneficial effects of continues endurance exercise compared to HIT[26].

Whether HIT causes the same mitochondrial adaptations as those seen in healthy humans has only been researched by two studies. A first study (without control group) demonstrated increased mitochondrial content (citrate synthase activity) and improved 24h blood glucose profile (measured 48-72h after last training) in type 2 diabetes mellitus after 2 weeks of HIT[27]. In another study a similar improvement in citrate synthase was observed in overweighed women using HIT in a fed or a fasted state[28].

Based on the data above, it is clear that HIT can have metabolic effects through muscular mitochondrial adaptations in healthy humans, and therefore is of great clinical relevance for people with or at risk for type 2 diabetes mellitus. Though, it is not yet clearly known what the effects of HIT are on muscular adaptations in patients at risk for type 2 diabetes mellitus. This study consequently tries to give an appropriate answer on the following research question: Does a 10 week-HIT program compared to a continuous aerobic training program (with same frequency, time per session and total volume) cause specific muscular mitochondrial adaptations in benefit of glycaemia control in patients at risk for type 2 diabetes mellitus?

Material and methods

The study was approved by the ethics committee of the Ghent University Hospital (September 2014; B670201318620). The study was registered at clinicaltrials.gov (NCT02798666). A signed informed consent was provided by all participants before study admission.

Participants (Figure 1)

Sixteen adult males at risk for diabetes type 2 (BMI-range 28-36 kg/m²) with an age range of 42 until 57 years and

Table 1. Details of the two different exercise training modes.

Training modus	Warming up (5')	Cycling (10')	Stepping or cycling (10')	Cycling (10')	Cooling down (5')
HIT	Stretching + Aerobic exercise lower and upper limb (30% of peak watt)	10 sprint bouts: 15" (100 rounds per minute) at 100% resistance at ventilatory threshold (week 1-5) increasing to 110% resistance at ventilatory threshold (week 6-10) + 45" (40-60 rpm) at 50% ventilatory threshold (week 1-5) increasing to 55% ventilatory threshold (week 6-10)	Continuous (60 rounds per minute) 100% of heart rate at ventilatory (week 1 to 5) and 110% of heart rate at ventilatory (week 6 to 10)	10 sprint bouts: 15" (100 rounds per minute) at 100% resistance at ventilatory threshold (week 1-5) increasing to 110% resistance at ventilatory threshold (week 6-10) + 45" (40-60 rounds per minute) at 110% resistance at ventilatory threshold (week 1-5) increasing to 110% resistance at ventilatory threshold (week 6-10)	Stretching + Aerobic exercise lower and upper limb (30% of peak watt)
CAT	5 min Idem HIT	continuous (60 rounds per minute) 100%of heart rate at ventilatory threshold (week 1 to 5) and 110% of heart rate at ventilatory threshold (week 6 to 10)	continuous (60 rounds per minute) 100% of heart rate at ventilatory threshold (week 1 to 5) and 110% of heart rate at ventilatory threshold (week 6 to 10)	continuous (60 rounds per minute) 100% of heart rate at ventilatory threshold (week 1 to 5) and 110% of heart rate at ventilatory threshold (week 6 to 10)	5 min Idem HIT
HIT: high intensity training; CAT: continuous aerobic training; rpm: rounds per minute.					

HbA1c lower than 6,5% were recruited and randomized in two experimental groups: high intensity interval training (HIT, n=8) and continuous aerobic training (CAT, n=8). A stratified randomization was performed (stratification based on age and BMI) using the envelope method (after stratification two participants with the same age and BMI had to choose an envelope to be allocated to CAT or HIT). Participants were included when they were sedentary (<60 min structured exercise/week) and excluded if they had diabetes (HbA1c >6,5%), severe musculoskeletal- (e.g. osteoarthritis), cardiovascular- (e.g. chronic heart failure) or respiratory (e.g. chronic obstructive pulmonary disease) problems, based on their medical files.

Intervention

Participants were enrolled in a 10 week HIT or a CAT protocol. Each session had duration of 40 minutes, and was performed twice a week, under supervision of two physiotherapists in the Physiotherapy Department of the University Hospital of Ghent (Table 1). The volume and frequency was equal between both training protocols. During the exercise training water *ad libitum* was available. Cycling was done on an Ergofit Cycle 400 and stepping on a Kettler Unix EX cross ergometer. All participants were asked to maintain normal physical activity and dietary patterns, and to refrain from exhaustive physical exercise three days prior and during the experimental period.

HIT-protocol

Each training session of the HIT protocol included a warming up (stretching of the large muscle groups and cardiovascular exercises at 30% of peak cycling power output) for five minutes, 10 minutes high intensity interval exercises, 10 minutes continuous aerobic exercises, 10 minutes high intensity interval exercises and a cooling down (stretching of the large muscle groups and cardiovascular exercises at 30% of peak Watt) for 5 minutes. Each high intensity part during the first 5 weeks, consisted of 10 bouts of 15 seconds at 100 rounds per minute at a cycling resistance matching with the ventilatory threshold, alternated with 45 seconds at 40-60 rounds per minute at 50% of the resistance matching with the ventilatory threshold. During HIT sessions, heart rate increased above 85% of peak heart rate. The intensity of the high intensity bouts increased with 10% from week 6 until week 10.

CAT-protocol

Each training session of the CAT protocol included a warming up (stretching of the large muscle groups and cardiovascular exercises at 30% of peak cycling power output) for five minutes, 3 continuous aerobic exercises of 10 minutes each, and a cooling down (stretching of the large muscle groups and cardiovascular exercises at 30% of peak cycling power output) for five minutes. During the continuous aerobic exercises of the protocol (cycling or stepping) participants exercised 3 times for 10 minutes at a heart rate similar to the heart rate
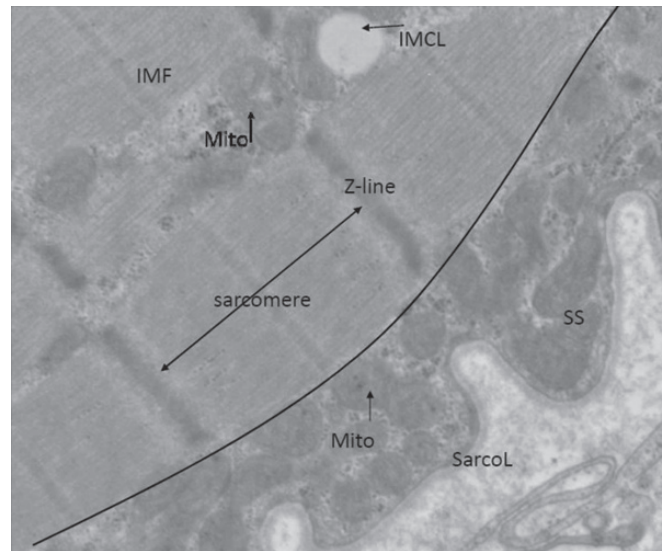
Figure 2. Transmission electron microscopy showing the presence of mitochondria and intramyocellular lipid droplets in the intramyofibrillar and subsarcollemal space of a biopsy of the vastus lateralis muscle of males with overweigth or obesity participating in an exercise training program (continuous aerobic training (CAT) or high intensity training (HIT)).
A: Transmission electron microscopy picture showing mitochondria (mito) and intramyocellular lipid droplets (IMCL) in the intramyofibrillar (IMF) and subsarcollemal (SS) space. The space between 2 Z-lines is the sarcomere. The black line is an imaginary line between IMF and SS. The space below the sarcolemma (SarcoL) is the SS. (x12.000 original magnification).

at ventilator threshold at a speed of 60 rpm. The first and third part of 10 minutes consisted of cycling, while the second part consisted of stepping. After 10 minutes of cycling, participants continued immediately with stepping. The intensity of CAT increased with 10% from week 6 until week 10.

Outcome variables

Outcome variables were measured, each time in the morning spread over 7 days before and after the 10 week training protocol. The general characteristics and maximal exercise test were measured 5 days prior to the start and 1 day after the training period. Basal respiratory exchange ratio and insulin sensitivity (oral glucose tolerance test) were measured after an overnight fasting, 3 days before the start and 3 days after the last training session. Basal respiratory exchange ratio was measured before the oral glucose tolerance test. The muscle biopsies were taken after an overnight fasting, 2 days prior to the start and 4 days after the last training session of the protocol. Prior to all measurements, participants were well informed and familiarized with the equipment and testing protocols. The quantification of all examined variables was performed by blinded assessors. All tests and measurements were conducted at the exercise laboratory of the Physiotherapy Department, Ghent University (maximal exercise test, basal respiratory exchange ratio), at the Department of Endocrinology University Hospital Ghent (oral glucose

tolerance test and muscle biopsy) or at the Department of Pathological Anatomy (muscle histology).

General characteristics

Height, Weight and BMI. Height was measured to the nearest 0.1 cm using a stadiometer (Holtain Ltd, Pembrokeshire, UK). Weight was measured to the nearest 0.1 kg on a digital balance scale (Seca, Germany) with the subject wearing lightweight clothing and no shoes. The body mass index (BMI) was calculated from weight and height (BMI= (weight (kg)/(height (m))^2).

Maximal cardiopulmonary exercise test. Participants were tested on a computer-driven cyclo-ergometer (Marquette Case, Marquette Electronics, Milwaukee, WI, USA) using a ramp protocol (20 W/min) starting at 40Watt. Twelve-lead electrocardiogram and heart rate were recorded continuously during the test, whereas blood pressure was measured with a manual sphygmomanometer every two minutes. Subjects were asked and encouraged to perform exercise testing until physical exhaustion. Tests were classified as maximal when respiratory exchange ratio increased above 1.1, heart rate increased above 80% of predicted heart rate (220-age) and/or plateau in oxygen uptake. Respiratory gas measurements were obtained using a Metalyzer 3B (Cortex, Leipzig, Germany). Oxygen consumption (VO2), carbon dioxide production (VCO2) and minute ventilation (VE) were measured continuously. Peak heart rate was expressed as the highest HR. Peak VO2 was

Table 2. Anthropometric data and exercise capacity of the participants at baseline and after the training period for each intervention group.

Parameters	CAT pre	CAT post	HIT pre	HIT post	P-value (interaction effects)
Age (year)	46.0 (5.6)	46.0 (5.6)	47.0 (3.4)	47.0 (3.4)	0.92
Height (cm)	174.5 (4.0)	174.5 (4.0)	175.8 (6.8)	175.8 (6.8)	0.99
Weight (kg)	101.6 (16.7)	100.4 (16.7)*	98.7 (12.6)	97.6 (13.2)*	0.73
BMI (kg/m^2)	33.0 (5.7)	32.6 (5.7)*	31.9 (5.6)	31.3 (5.7)*	0.88
Respiratory exchange ratiomax	1.16 (0.05)	1.15 (0.08)	1.17 (0.06)	1.16 (0.05)	0.85
Peak VO2 (l/min)	2.9 (0.6)	3.0 (0.6)	2.9 (0.6)	3.1 (0.5)#	0.004
Rel.peakVO2 (ml/kg/min)	30.3 (9.4)	30.5 (9.8)	30.9 (7.5)	32.9 (7.2)#	0.011
Peak cycling power output (watt)	218 (57)	228 (60)*	240 (50)	274 (50)#	<0.001
Peak heart rate (beats/min)	163 (19)	162 (19)	163 (16)	161 (18)	0.41
VO2 at ventilatory threshold (l/min)	2.3 (0.5)	2.4 (0.5)*	2.2 (0.5)	2.6 (0.5)#	0.009
Cycling Power output at ventilatory threshold (Watt)	171 (46)	173 (45)	168 (43)	206 (50)#	0.002
HR at ventilatory threshold (beats/min)	139 (15)	135 (13)	140 (23)	145 (20)	0.10

Data are expressed as mean (standard deviation). Significance level is set at P<0.05. Data were statistically evaluated by repeated measures ANCOVA (covariate OGTT) post hoc Bonferroni. Interaction effects are mentioned in the last column (P-value).The post hoc Bonferroni to evaluate time effects within each group are mentioned by * and #. *= P<0.05: CATpre versus CATpost; #= P<0.05: HITpre versus HITpost. // BMI= body mass index. PeakVO2= oxygen consumption at peak level; relpeakVO2 = oxygen consumption at peak level expressed per body weight.

Table 3. Basal respiratory exchange ratio and insulin sensitivity of the participants at baseline and after the training period for each intervention group.

Parameters	CAT pre	CAT post	HIT pre	HIT post	P-value (between group)
Basal respiratory exchange ratio	0.82 (0.03)	0.84 (0.04)	0.85 (0.03)	0.79 (0.04)#	<0.001
Fasting plasma glucose(mg/dl)	99.1 (11.2)	99.5 (9.3)	102.1 (8.8)	96.4 (10.7)	0.117
Fasting plasma insulin (µU/ml)	15.8 (5.4)	15.7 (7.9)	21.0 (5.4)§	14.3 (8.5)#	0.002
Area under the curve of glucose (mg/dl)	143.9 (30.6)	137.0 (23.7)	191.1 (32.6)§	152.1 (39.7)#	<0.001
Area under the curve of insulin (µU/ml)	184.9 (41.5)	179.4 (40.4)	286.1 (96.1)§	197.1 (82.8)#	<0.001

Data are expressed as mean (standard deviation). Significance level is set at P<0.05. Data were statistically evaluated by repeated measures ANCOVA (covariate OGTT) post hoc Bonferroni. Interaction effects are mentioned in the last column (P-value).The post hoc Bonferroni to evaluate time effects within each group are mentioned by * and #. §=P<0.05: CATpre versus HITpre; *= P<0.05: CATpre versus CATpost; #= P<0.05: HITpre versus HITpost.

expressed as the highest attained VO2 during the final 30 seconds of exercise according to the American Thoracic Society guidelines. The ventilator threshold was determined based on the metabolic equivalents of O2 and CO2[27].

Basal respiratory exchange ratio

Participants had to lie in a supine position for 30 minutes after an overnight fast in a quiet and thermo-neutral environment. Basal oxygen consumption and carbon dioxide production was measured with an automated respiratory gas analyzer using a mask (Metalyzer 3B; Cortex, Leipzig, Germany). Respiratory exchange ratio was calculated as

VCO2/VO2. Data were gathered from the last 20 minutes when a stable phase was reached.
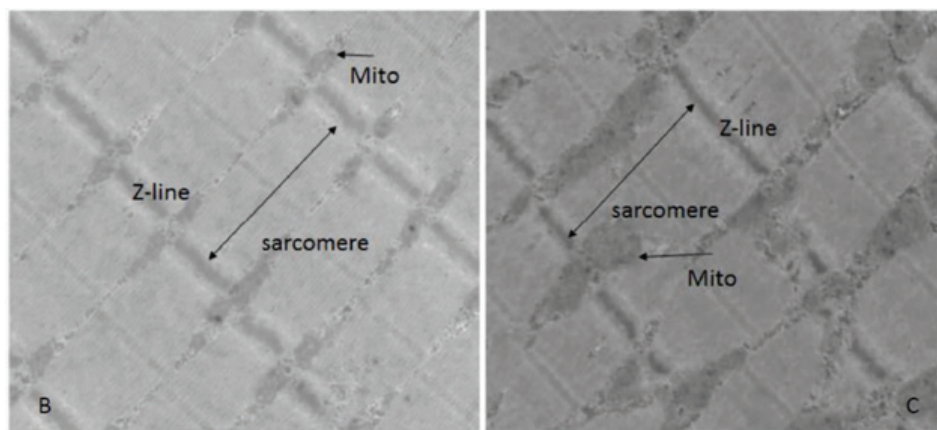
Oral glucose tolerance test

At 8:00 A.M., after a 10- to 12-h overnight fast, patients were administered a 75 g glucose solution (200 ml) and had to drink this within 5 minutes. Blood serum samples were taken after 30, 60, 90 and 120 min. Blood samples were centrifuged and stored at -80°C within 30 min after collection. Insulin levels were determined using the immunoanalyzer COBAS e411 (Roche) which had an intra-coefficient of variation between 2.93% (µ=14.16 µU) and 2.38% (µ=62.6

Table 4. Number (n) and surface (s) of lipid droplets and mitochondrial subsarcollemal and intramyofibrillar site of the participants at baseline and after the training period for each intervention group.

	CATpre (n=8)	CATpost (n=8)	HITpre (n=8)	HITpost (n=8)	P-value (interaction effects)
Intramyofibrillarlipid (number)	40 (24)	53 (33)	79 (52)	154 (101)#	0.002
Subsarcollemallipid (number)	13 (2)	12 (2)	13 (10)	20 (14)#	0.007
IntramyofibrillarMito (number)	130 (12)	127 (12)	106 (23)	182 (46)#	<0.001
SSmito (n)	63 (20)	63 (15)	40 (16)§	82 (25)#	0.001
Intramyofibrillarlipid (su)	21.7 (13.6)	31.1 (20.7)	24.4 (15.7)	51.5 (20.9)#	0.043
Subsarcollemallipid (surface)	6.8 (2.1)	7.7 (2.6)*	5.7 (3.0)	6.9 (3.3)#	0.716
IntramyofibrillarMito (su)	21.7 (3.4)	20.8 (2.3)	17.6 (7.0)	25.7 (7.3)#	0.002
Subsarcollemallipid (surface)	12.7 (4.0)	12.4 (2.6)	6.0 (2.7)§	15.2 (8.5)#	0.008

Data are expressed as mean (standard deviation). Significance level is set at $P<0.05$. Data were statistically evaluated by repeated measures ANCOVA (covariate OGTT) post hoc Bonferroni. Interaction effects are mentioned in the last column (P-value). Baseline differences between HIT and CAT are mentioned by §: $p<0.05$. The post hoc Bonferroni to evaluate time effects within each group are mentioned by * and #. *= $P<0.05$: CATpre versus CATpost; #= $P<0.05$: HITpre versus HITpost.

Figure 2B and C. Transmission electron microscopy picture showing mitochondria (mito) in the intramyofibrillar (IMF) space. The space between 2 Z-lines is the sarcomere. Picture B is a muscle sample before the start of HIT. Picture C Is a muscle sample after 10 weeks of HIT showing larger surface area. (x12.000 original magnification).

μU) for insulin. Glucose was analysed by the hexokinase method (COBAS, Roche) and its intra-coefficient of variation ranged between 1.58% (μ=64.7 mg/dl) and 1.38% (μ=369 mg/dl). To evaluate insulin sensitivity oral glucose tolerance test-composite score was calculated based on the reference of Matsuda et al.[29]. This score is composed as 10,000/square root of (fasting glucose x fasting insulin) x (mean glucose x mean insulin during oral glucose tolerance test). The higher the score, the better the insulin sensitivity. Also area under the curve of insulin and glucose was calculated using the trapezoidal rule, and compared. First the trapezoidal rule was applied to calculate four 30 min blocks (0'-30', 30'-60', 60'-90' and 90'-120'); subsequently the sum was made of those blocks to define the total area under the curve.
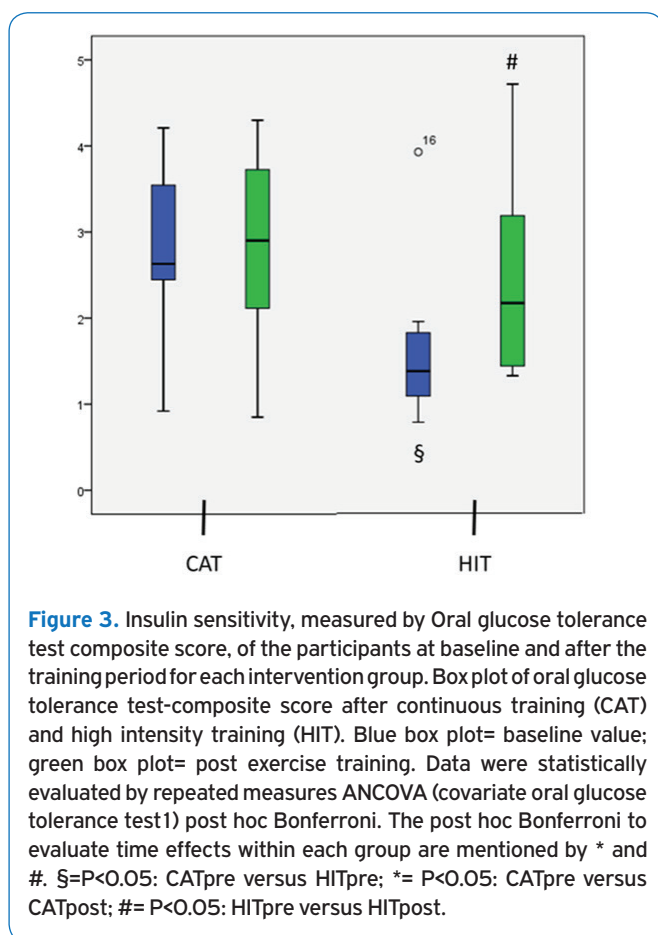
Muscle biopsy

After a 1-h rest period, a percutaneous needle biopsy sample was taken from the right vastus lateralis muscle with a biopsy gun (Bard Magnum, needle 12G) under local anaesthesia through a 2-mm incision in the skin (2-3 ml lidocaine) under the guidance of ultrasound. With this technique the sample is aspirated and remains in the branula until it is extracted. The sample is taken out as a whole and not fragmented. Two muscle samples of 20 mg were taken and incubated in 4% Paraformaldehyde and 5% Glutaraldehyde in 0,1 M cacodylate for transmission electron microscopy. Muscle tissue was then fixated by staining with osmium tetroxide and dehydrated with ethanol and propylene oxide. Then the muscle tissue was embedded

Table 5. Association between changes in metabolic, physical and histological parameters in the total group (combined CAT and HIT).

(n=16)	Delta oral glucose tolerance test (r-value)	P-value
deltapeakVO2	0.474	0.06
deltarelpeakVO2	0.436	0.09
Delta respiratory exchange ratio	-0.740	0.001
Delta intramyfibrillar lipid (number)	0.294	0.27
Delta intramyofibrillar lipid (surface)	0.300	0.26
Delta subsarcollemal lipid (number)	0.318	0.23
Delta subsarcollemal lipid (surface)	0.172	0.52
Delta intramyofibrillar mitochondria (number)	0.462	0.07
Delta intramyofibrillar mitochondria (surface)	0.540	0.031
Delta subsarcollemal mitochondria (number)	0.540	0.031
Delta subsarcollemal mitochondria (surface)	0.364	0.08

Data were statistically evaluated by Pearson correlation coefficients (r-value). Significance level is set at P<0.05. Delta= pre-post of each parameter. PeakVO2 = oxygen consumption at peak level; relpeak VO2 = oxygen consumption at peak level expressed per body weight.

Figure 3. Insulin sensitivity, measured by Oral glucose tolerance test composite score, of the participants at baseline and after the training period for each intervention group. Box plot of oral glucose tolerance test-composite score after continuous training (CAT) and high intensity training (HIT). Blue box plot= baseline value; green box plot= post exercise training. Data were statistically evaluated by repeated measures ANCOVA (covariate oral glucose tolerance test1) post hoc Bonferroni. The post hoc Bonferroni to evaluate time effects within each group are mentioned by * and #. §=P<0.05: CATpre versus HITpre; *= P<0.05: CATpre versus CATpost; #= P<0.05: HITpre versus HITpost.

glass microtome (Ultramicrotomy system, Pyramitome, LKB (Stockholm-Bromma, Sweden). After a tissue orienting staining with toluidine blue, the semi-thin sections were cut into thin longitsections (70 nm) with a diamond microtome (Reichert Supernova, Leica). Sections were then stained with uranylacetate (to stain RNA, ribosomes, mitochondria, membrane) and lead nitrate (to stain filaments and lipid droplets) and finally covered with carbon. Transmission electron microscopy was used to determine intramyocellular lipid content and mitochondrial characteristics. Samples were viewed at 6,500× using a JEOL 1200EX TEM. Sixteen micrographs were acquired from 8 randomly sampled longitudinal sections of muscle fibres (2 micrographs/fibre) from each individual muscle - one micrograph acquired near the cell surface representing the subsarcolemmal region and the other acquired of parallel bundles of myofibrils representing the intramyofibrillar region (Figure 2A and 2B). Lipid droplets and mitochondrial fragments were circled and converted to actual size using a calibration grid. For each set of 16 images, mean intramyocellular lipid content or mitochondrial size (μm^2), total number of intramyocellular lipid content droplets or mitochondria per square micrometer of tissue ($\#/\mu m^2$) were calculated in the intramyofibrillar and subsarcolemmal compartments by digital imaging software (Image Pro Plus, ver. 4.0; Media Cybernetics, Silver Springs, MD). The reference for subsarcolemmal space quantification was the cytoplasmic space between the sarcolemma and the first layer of myofibrils.

Statistical analyses

All data were analyzed with a commercially available statistical software program (Statistical Package for the Social Sciences, SPSS 20.0, SPSS Chicago, IL, USA). Normality of the data was tested with the Shapiro-Wilk test and scatter plots. Data are expressed as mean ± standard deviation. An

in capsules with the addition of Epon, another fixative. After hardening the tissue overnight in an oven at 60°C, the tissue was cut into longitudinal semi-thin sections (4 μm) with a

independent t-test was used to evaluate baseline differences. To evaluate interaction and time effects of HIT compared to CAT a repeated measures ANCOVA (group*time) with post hoc Bonferroni was used. Oral glucose tolerance test was used as a covariate in the ANCOVA, as this parameter was significantly different at baseline between both groups. Significance levels were set at P<0,05 for all tests.

Results

General data on compliance and on adverse events of the participants

Attendance to the training protocol ranged between 18 to 20 sessions, showing the compliancy of the participants. Only 2 participants in the CAT group had 18 (CAT1; work circumstances) or 19 sessions (CAT2; sickness) and in the HIT group only 1 participant had 18 sessions (HIT1; injury). During the training sessions no adverse events were reported.

Baseline characteristics of the participants

Baseline characteristics of the participants are shown in Tables 2, 3 and 4 (CATpre and HITpre). Groups did not differ in age, length, weight, BMI, peakVO2, relative peakVO2, peak heart rate, peak wattage, Respiratory exchange ratio, basal glucose levels and basal insulin levels. Insulin sensitivity, expressed by area under the curve glucose (p=0.010) and area under the curve insulin (p=0.016) and oral glucose tolerance test score (p=0.040), were significantly higher at baseline in the CAT group compared to the HIT group. At the level of the muscle histology, no baseline differences in intramuscular lipid at the intramyofibrillar region or subsarcollemal (p>0.05) were noticed. Mitochondrial content expressed as number or surface area at the subsarcolemmal region was significant higher in the CAT group compared to the HIT group (p=.002), while no difference was seen between both groups in baseline mitochondrial content at the intramyofibrillar region.

Effect of exercise training on anthropometric data and exercise capacity (Table 2)

Ten weeks of training significantly reduced the weight and BMI of the participants, independently of the type of training (p=0.016 respectively p=0.007). Peak VO2 and peak power (Wattage) significantly changed over time (p=0.023 respectively p<0.001) reliant on the exercise training type (time*group: p=0.004 respectively p<0.001). Post-hoc Bonferroni corrections demonstrated a significant increased peak VO2 after 10 weeks of HIT (p=0.023), while the peak VO2 even slightly decreased after 10 weeks of CAT. Peak power increased more after 10 weeks of HIT compared to CAT (post-hoc Bonferroni p<0.001).

Effect of exercise training on basal respiratory exchange ratio and insulin sensitivity (Figure 3; Table 3)

Ten weeks of training did not significant influence basal glucose levels over time, nor between the groups. Basal insulin levels, on the other hand, significantly changed over time (p=0.001) and reliant the type of training (time * group: p<0.002), with more profound decrements of basal insulin levels after HIT compared to CAT (p<0.001). Insulin sensitivity improved over time and dependent of the type of training (time*group), expressed by the area under the curve glucose (P<0.001), area under the curve insulin (P<0.001). Post hoc corrections demonstrated more profound decreases in area under the curve glucose and area under the curve insulin 10 weeks post HIT compared to CAT (p<0.01). Furthermore, significantly increased oral glucose tolerance test composite scores of the participants were detected (p=0.003), dependently of the type of training (time * group: p=0.005). Post hoc Bonferroni corrections demonstrated an increased oral glucose tolerance test composite score after 10 weeks of HIT (p=0.003), but not after CAT.

Basal respiratory exchange ratio is influenced depending from the type of training (time * group) (p<0.001). While basal respiratory exchange ratio decreased significantly after 10 weeks of HIT (p=0.001), no significant changes were detected in respiratory exchange ratio after 10 weeks of CAT (p=0.37).

Effect of exercise training on muscle histology

At the intramyofibrillar site, lipid content and surface area significantly increased after 10 weeks of training depending on the type of training (time*group) (p=0.002 respectively p=0.043), with larger positive effects after HIT (number: p<0.001; surface area: p<0.001) compared to the CAT group. At the subsarcolemmal site, lipid *surface area* significantly increased over time (p=.002), independently of the type of training. Lipid content significantly changed after 10 weeks of training depending on the type of training (time*group) (p=0.007), with positive increments after HIT (number: p<0.001) compared to the CAT group.

At the intramyofibrillar site, mitochondrial *content* and *surface area* significantly increased after 10 weeks of training (Figure 2A and 2B) depending on the type of training (time*group) (p<.001 respectively= 0.002) with larger positive effects after HIT (number: p<0.001; surface area: p=0.001) compared to CAT. At the subsarcolemmal site, mitochondrial *content* and *surface area* significantly increased after 10 weeks of training depending on the type of training (time*group) (p=0.001 respectively p=0.008), with increased intramuscular mitochondrial content after HIT, but not after CAT at both sites (number: p=0.004; surface area: p=0.001).

Associations between changes in oral glucose tolerance test scores and changes in basal respiratory exchange ratio and changes in muscle histology

Bivariate Pearson correlations revealed a significant negative association with changes in basal respiratory exchange ratio (r=-0.740; p=0.01). Positive associations between changes in oral glucose tolerance test and changes

in muscle histology were noticed for mitochondrial content (intramyofibrilar: r=0,462; p=0.071 and subsarcolemmal: r=0.540; p=0.031) and surface (intramyofibrilar: r=0.540; p=0.031 and subsarcolemmal: r=0.364; p=0.083).

Discussion

This study showed that 10 weeks of HIT is more effective to improve insulin sensitivity compared to continuous aerobic training in patients at risk for type 2 diabetes mellitus. These changes are accompanied with an increased muscle mitochondrial content and a decreased basal respiratory exchange ratio, indicating an optimized fat oxidation, and an increased aerobic capacity.

This study supports the evidence that a small volume of exercise performed at a high intensity can elicit significant skeletal muscle adaptations in view of mitochondrial content and lipid metabolism.

The results of the current study, obtained in overweight and obese males are in line with previous research in which the beneficial effects of HIT on aerobic capacity, expressed as peakVO2 and ventilatory threshold, in athletes and young healthy participants[30], in middle-aged and older patients[31], as well as in people with chronic disease such as chronic obstructive pulmonary disease[32], coronary heart disease[33], type 2 diabetes mellitus[34,35] and people at risk for diabetes (overweight and obesity, metabolic syndrome, prediabetes)[23,36] were demonstrated. The effects of the continuous aerobic training program used in this protocol, however, are less clear compared to previous published data. The American College of Sports Medicine guidelines recommend an engagement in moderate-intensity cardiorespiratory exercise training for ≥30 minutes/day on ≥5 days/week for a total of ≥150 minutes/week, vigorous-intensity cardiorespiratory exercise training for ≥20 minutes/day on ≥3 days/week (≥75 minutes/week), or a combination of moderate- and vigorous-intensity exercise to achieve a total energy expenditure of ≥500-1000 Metabolic Equivalent of Task (MET)·minutes/week[37]. Our participants only trained 30 to 40 minutes, twice a week during 10 weeks at an initially load of 100% ventilatory threshold which increased after 5 weeks to 110% ventilatory threshold. It was however the purpose to compare two different modes with the same total volume, duration per session and frequency.

Our study also observed a large effect of HIT on insulin sensitivity measured by area under the curve of glucose and insulin and the oral glucose tolerance test composite score compared to CAT, which is in line with other studies[18-21]. A possible role of mitochondrial content and function in the amelioration of the insulin sensitivity can be mentioned. Phielix[38] investigated the effects of a 12 week combined endurance and strength exercise program on *ex vivo* mitochondrial content and the association with insulin sensitivity (hyperinsulinaemic-euglycaemic clamp) in 15 type 2 diabetic and 14 non-diabetic matched control males. They concluded that diabetic patients could increase their mitochondrial content to the same extent as healthy individuals, which was strongly associated with improved insulin sensitivity.

In our study, we could also observe a significant increase in mitochondrial content (number and surface). Comparable mitochondrial content were reported for low-volume sprint interval training after 2[39], 6[11] and 12[28] weeks of training. Our data are also in line with the findings of MacInnis et al.[7] and Daussin et al.[40]. They also found superior mitochondrial adaptations in human skeletal muscle after HIT compared to continuous training. This superior mitochondrial adaptation was reflected in higher citrate synthase content. They hypothesized that the augmented response observed following HIT compared to continuous training was a result of greater cellular stress incurred by exercising at a higher intensity[7,40]. After bivariate correlation between the changes in oral glucose tolerance test and changes in muscle mitochondrial content, we could demonstrate positive associations with changes in mitochondrial content and surface. Due to the fact that there are changes in mitochondrial content and surface after HIT (significant increase), but not after CAT in our study group, this might partly explain the significant different outcome at the level of insulin sensitivity.

We could also reveal a significant negative association between changes in basal respiratory exchange ratio and changes in oral glucose tolerance test. This means when basal respiratory exchange ratio is decreasing, indicating a more efficient lipid utilization/oxidation, this is associated with an increased insulin sensitivity. In this study, we could also demonstrate a decrease in basal respiratory exchange ratio after HIT, but not after CAT. This result is in line with the findings of Sartor et al.[41], who investigated whether a carbohydrate-reduced diet in combination with HIT enhances insulin sensitivity and fat oxidation in obese individuals. They found significantly decreased respiratory exchange ratio (0,91 versus 0,88). When basal respiratory exchange ratio is decreased this indicates that fat oxidation is optimized. Alkahtani et al.[42] showed that HIT increased fat oxidation by alterations from high intensity to low intensity in overweight/obese men. In the continuous group of this study, effects were small which are in line with existing literature. Van Aggel-Leijssen et al.[43] investigated the effect of CAT at 40% of VO2max for 50 minutes for 12 weeks compared to 70% of VO2 max for 30 minutes for 12 weeks. Total fat oxidation did not change at rest in any group. Our training protocol was based on 100% to 110% VAT, which can be translated to intensity between 55 and 75% of VO2 max, for 30 minutes for 10 weeks.

At last, we found an increase of intramyocellular lipid content after HIT. There is only one study[44] focusing on intramyocellular lipid content, but they found no significant changes. A possible explanation can be that the exercise protocol was different to ours in exercise modality (a combination of interval training with resistance training) and in intensity (hit were bouts of 30 seconds of 50+-60% of Wattmax).The results of our CAT were comparable with Tarnopolsky et al.[45] and Bajpeyi et al.[46] who reported an equal or even a higher intramyocellular lipid content.

Strengths and weaknesses

All measurements taken in this study were performed by blinded assessors. The methods used and calculations from gathered data were done according to valid strategies (vetilatory threshold, oral glucose tolerance test composite score). The use of electron microscopy (giving insight in the lipid and mitochondrial histology) was done to give us direct (visual) information on the content and surface, and was conducted by experienced assessors and according to the most optimal guidelines.

Beside several strengths, this study has also some limitations. This study used two different exercise training protocols which have the same total volume and frequency but were not eucaloric. This was not our purpose, but in a following study it would be interesting to make the caloric expenditure of both interventions equal to each other. Then we can focus whether the mode of training (alternating high intensities with low intensities) results in different outcomes.

Due to practical problems (measuring in batches) we could not measure the insulin concentration immediately after oral glucose tolerance test. Therefore we were not able to randomize and match taken in account the oral glucose tolerance test outcome. However even after statistical correction of oral glucose tolerance test at baseline strong significant effects of HIT remained, which make the outcome of the study valid for clinical use.

The measurement of the substrate utilization and insulin sensitivity was done by respiratory exchange ratio at rest respectively oral glucose tolerance test. Furthermore, measuring substrate utilization during submaximal exercise (below anaerobic threshold) in future studies, could give a more in depth view of fat oxidation. Despite the fact that oral glucose tolerance test is widely used in practice and research, Ko et al.[47] reported low reproducibility. A hyperinsulinaemic-euglycaemic clamp should be a more reproducible alternative for research purposes in the future.

Conclusion

High intensity training has stronger beneficial effects on physical fitness, basal respiratory exchange ratio and insulin sensitivity compared to continuous aerobic training in overweight/obese men. The positive metabolic effects are accompanied by an increase in skeletal muscle mitochondrial content. This study indicates that low volume high intensity training can be used in the prevention of diabetes.

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