High-intensity interval training improves insulin sensitivity in older individuals

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Short title: High-intensity interval training improves insulin sensitivity

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Abstract

Aim: Metabolic health may deteriorate with age as a result of altered body composition and decreased physical activity. Endurance exercise is known to counter these changes delaying or even preventing onset of metabolic diseases. High-intensity interval training (HIIT) is a time efficient alternative to regular endurance exercise and the aim of this study is to investigate the metabolic benefit of HIIT in older subjects.

Methods: 22 sedentary male (n=11) and female (n=11) subjects aged 63 ± 1 years performed HIIT training three times/week for six weeks on a bicycle ergometer. Each HIIT session consisted of five 1 minute intervals interspersed by 1½ minute rest. Prior to the first and after the last HIIT session whole-body insulin sensitivity, measured by hyperinsulinaemic-euglycaemic clamp, plasma lipid levels, HbA1c, glycaemic parameters, body composition and maximal oxygen uptake were assessed. Muscle biopsies were obtained wherefrom content of glycogen and proteins involved in muscle glucose handling were determined.

Results: Insulin sensitivity (p=0.011) and maximal oxygen uptake increased (p<0.05) in both genders while plasma cholesterol (p<0.05), low density lipoprotein (p<0.05), visceral fat mass (p<0.05) and percent body fat (p<0.05) decreased after six weeks HIIT. HbA1c decreased only in males (p=0.001). Muscle glycogen content increased in both genders (p=0.001) and in line GLUT4 (p<0.05), glycogen synthase (p=0.001) and hexokinase II (p<0.05) content all increased.

Conclusion: Six weeks of HIIT significantly improves metabolic health in older males and females by reducing age related risk factors for cardio-metabolic disease.

Keywords

Aging, body composition, glucose metabolism, high-intensity interval training, insulin sensitivity, skeletal muscle.

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Introduction

The process of aging includes biological changes that lead to decline in physical function and metabolic health. Sarcopenia and increased abdominal fat, which accounts for part of these changes\(^1\text{--}^4\), have been associated with reduced insulin sensitivity seen with aging\(^4\text{--}^7\). The origin of these alterations is not fully known but a drop in physical activity is strongly suggested as the main factor leading to reduced muscle mass and increased obesity, finally reducing insulin sensitivity\(^8\text{, }^9\).

Traditional endurance training in the form of moderate-intensity continuous exercise counteracts, at least partly, the changes observed with age, by inducing beneficial changes in body composition, glycaemia, dyslipidaemia and blood pressure\(^10\text{, }^11\). However, it seems difficult to fit time consuming endurance training into a busy schedule of daily life and lack of time, motivation and convenience are often put forward as major reasons not to exercise\(^12\text{--}^15\).

High-intensity interval training (HIIT), an alternative to endurance training, is based on low-volume training at high intensity and is thus more time efficient. The effect of HIIT has been investigated in both young and older subjects in several studies and overall HIIT is as effective as moderate intensity endurance training to increase maximal oxygen uptake (VO\(_2\text{max}\)) and muscle mass and reduce fat mass, including visceral fat\(^16\text{--}^22\). The effect of HIIT on insulin sensitivity has, to our knowledge, only been measured in three studies by the gold standard, the hyperinsulinaemic-euglycaemic clamp showing no change in young females\(^20\) or an increase in both young and older males and females\(^23\text{, }^24\) in whole-body insulin sensitivity.

Though some studies have demonstrated that older people can complete a HIIT intervention with various improvements in metabolic health\(^17\text{, }24\text{--}26\), the current knowledge of the effects of HIIT in older subjects is limited. It is further unclear if the response to HIIT is gender specific since HIIT studies including both males and females often report pooled effects\(^16\text{, }21\text{, }25\). However, some studies have found differential gender responses to HIIT showing a higher reduction in body fat and indicated improved insulin sensitivity (Cederholm index) in males and increased VO\(_2\text{max}\) in females\(^27\text{, }28\).

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In the present study we evaluated the gender specific effect of six weeks HIIT on insulin sensitivity in a population of 55-75 year old, sedentary males and females. We further assessed the influence of HIIT on glucose metabolism in muscle as well as risk factors for metabolic disease. Based on prior studies we hypothesized that whole-body insulin sensitivity and muscle glucose metabolism would improve in response to six weeks HIIT. Moreover, we hypothesized that HIIT would reduce body fat mass.

Results

Compliance

18 subjects completed the 18 planned HIIT sessions while three male subjects completed 17 and one female 16 sessions during the six weeks HIIT intervention. To validate that the subjects trained at high and comparable intensities, the respiratory exchange ratio (RER) (ratio of CO₂ produced and O₂ used) and maximal heart rate (HR) were measured during the 5ᵗʰ HIIT interval and HR relative to maximal HR measured at VO₂max was calculated (HR%) at session 6 (RER = 1.02 ± 0.01, HR = 156 ± 3, HR% = 98 ± 2), 12 (RER = 1.03 ± 0.02, HR = 156 ± 4, HR% = 98 ± 2) and 18 (RER = 1.03 ± 0.02, HR = 153 ± 4, HR% = 96 ± 2). The average RER-value and HR of the three sessions did not differ between genders. Males and females trained at a similar load corresponding to 124 ± 3% of their individual watt max at session 2-6 and 135 ± 3% at session 7-20.

Body composition

Subject characteristics are shown in table 1. As expected percent whole body fat, android and gynoid fat distribution, lean body mass and leg lean mass differed significantly between genders. Body weight did not change in response to the HIIT intervention, but significant reductions in percent body fat and visceral fat mass (p<0.05), android (p<0.01) and gynoid (p<0.001) fat distribution and a borderline reduction in fat mass (p=0.053) were observed as main effects. Lean body mass, leg lean

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mass and leg fat mass were not altered as well as hip and waist circumference, waist-hip ratio, thigh volume and estimated mass of m. quadriceps femoris remained unchanged.

Lipid and inflammatory parameters

Basal concentrations of plasma FFA, glycerol, cholesterol, HDL and LDL were significantly higher in females compared to males (table 1). Plasma FFA, glycerol, HDL, triglyceride and high-sensitive C-reactive protein (hsCRP) concentrations did not change (main effect) in response to HIIT training, but a significant interaction was present for plasma HDL which decreased slightly in females (p=0.05). Total plasma cholesterol and LDL levels were significantly reduced (main effect, P<0.05) after six weeks HIIT (table 1).

Glucose metabolism

Whole-body insulin sensitivity improved significantly (p=0.011) in response to HIIT (figure 2). The whole-body insulin sensitivity did not differ between genders, but a borderline significant interaction (p=0.068) indicated an increase in males (p=0.004) and not in females (p=0.53).

Before and after the HIIT intervention whole-body insulin sensitivity correlated similarly and significantly with visceral fat mass (R=-0.70, p=0.002) and (R=-0.69, p=0.002), respectively (figure 3).

Fasting plasma glucose, insulin, C-peptide and area under the curve (AUC) of plasma glucose and insulin did not differ between gender and no changes were found in response to HIIT (table 1 and 2). A significant interaction (p<0.001) showed that HbA1c decreased significantly in males, but was unchanged in females (table 1). Insulin secretion rate (ISR) during the IVGTT was higher in females compared to males (p<0.01) (table 2) and did not change with the intervention.
In response to HIIT protein content of GLUT4 (p<0.05), glycogen synthase (p=0.001) and hexokinase II (p<0.05) increased (figure 4) as well as muscle glycogen content (p<0.001) (table 3). Expression of SNAP23 decreased in response to HIIT (p<0.05) while a higher content was present in females compared to males (p=0.004) (figure 4). Glycogen phosphorylase content was not changed (figure 4).

Aerobic fitness parameters

VO₂max was higher in males compared to females, and increased after HIIT in both genders (p<0.05) (table 3). Maximal load (watt) and time to fatigue (TTF) were higher and longer, respectively, in males than females. Significant interactions showed that maximal load was increased only in males (p<0.001), while TTF improved in both genders, but significantly more in males compared to females (table 3). Muscle CS activity (p<0.001) increased in response to HIIT (table 3).

Diet registration

Before the HIIT intervention the daily macronutrient intake was 37.8 ± 1.5% fat, 16.4 ± 0.6% protein, 42.5 ± 1.4% carbohydrate and 3.3 ± 0.9% alcohol and in the days around HIIT session 10 the macronutrient intake was 37.8 ± 1.9% fat, 17.3 ± 0.9% protein, 40.9 ± 2.0% carbohydrate and 4.0 ± 1.1% alcohol.

The energy intake was higher in males than females (p<0.05) and a borderline significant higher percent fat intake was found in females (p=0.053). The subjects habitual nutrition intake did not change significantly during the study (Pre: 8715 ± 490 KJ/day; HIIT session 10: 8726 ± 554 KJ/day; n=19, p=0.98).

Discussion

This study demonstrates that sedentary older males and females improve several indicators of metabolic health combined with a higher VO₂max in response to six weeks HIIT training. First of all, the whole-body insulin sensitivity increased as a main effect while HIIT induced a decrease in HbA1c in the male subjects.

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In addition, subjects also experienced reduced plasma total cholesterol and LDL cholesterol levels as well as loss of total percent body fat and visceral fat mass which emphasizes the metabolic benefit. Importantly, the training modality was well tolerated by all subjects and there were no drop-outs due to adverse effects of the training. Thus, we show that HIIT can be applied with significant favourable metabolic effects to older sedentary subjects.

Several of the HIIT induced metabolic improvements assessed in the present study are also observed in response to endurance training. Different studies evaluating the effect of endurance training in older obese subjects have reported improved VO\textsubscript{2}max and insulin sensitivity and reduced body fat mass, visceral fat mass and LDL in both genders \textsuperscript{11,30-32}. These data thus emphasizes that HIIT can be applied as an alternative to endurance training with similar metabolic benefits, also to older obese subjects.

Exercise has repeatedly been demonstrated to have a positive effect on insulin sensitivity in both young and older subjects \textsuperscript{11,25,33,34}. However, to our knowledge whole-body insulin sensitivity has only been measured once by clamp in older subjects performing HIIT \textsuperscript{24}, and in young subjects only two studies are available at present \textsuperscript{20,23}. Robinson et al. \textsuperscript{24} found an increase in insulin sensitivity in young and older males and females after 12 weeks HIIT, three times a week, however combined with 45 minutes incline walking twice a week. In young males and females Richards et al. \textsuperscript{23} did also report an increase in insulin sensitivity after 6 HIIT sessions while Arad et al. \textsuperscript{20} found no change in young overweight and obese women after 14 weeks HIIT, three times a week. Our finding of improved insulin sensitivity in response to HIIT is supported by most \textsuperscript{28,35-37}, but not all \textsuperscript{38,39} studies, which have applied surrogate measures of insulin sensitivity using different indexes (HOMA, Cederholm or Matsuda) in young or middle aged healthy males and females. The HIIT protocols applied in the above referenced studies vary in intensity and duration and there is also a variation in age, baseline metabolic condition and activity level of the subjects. Despite these differences it is
strongly indicated that HIIT enhances insulin sensitivity in young subjects, and the present study emphasize that the effect of HIIT is independent of age.

The HIT induced improvement in insulin sensitivity shown in the present study was not gender specific which supports the findings of Robinson et al.\textsuperscript{24} and Richards et al.\textsuperscript{23}. Constrastingly, Metcalfe et al.\textsuperscript{28} showed an improvement in insulin sensitivity (Cederholm index) in males only, while Arad et al.\textsuperscript{20} showed no effect in females. The number of studies evaluating the effect of HIIT on insulin sensitivity in both genders is low and further investigations are required.

The gender specific difference in the improvement in insulin sensitivity observed in prior studies could be explained by a difference in the training intensity. In our study, the training was supervised and the observation of mean R values above 1.0 at three HIIT sessions (6\textsuperscript{th}, 12\textsuperscript{th} and 18\textsuperscript{th}) show that the training intensity was identically high both across sessions and gender and thus true HIIT as planned. In addition, the males and females trained at a similar percent load of their individual watt max. Finally, the improvement in VO\textsubscript{2}max and CS activity implies that the training load was adequate to stimulate the classical training effects in both genders.

Whole-body insulin sensitivity largely depends on the glucose uptake, metabolism and storage capacity in skeletal muscle. We found increased protein expression of several key proteins and enzymes in skeletal muscle glucose handling. First, GLUT4 content increased with HIIT, which is in line with previous findings in both young and older subjects\textsuperscript{26, 35, 40}. This was combined with an increase in the protein content of two key enzymes, hexokinase II and glycogen synthase, and in stored glycogen content in the muscle, which is in accordance with Perry et al.\textsuperscript{40} reporting increased glycogen content in muscle in young subjects performing HIIT. We found no alteration in glycogen phosphorylase protein content, which indicates that the muscle favours storage of glucose rather than oxidation as an adaptation to HIIT.
A novel observation in the present study was the higher SNAP23 muscle content in females than males and a reduction with HIIT in both genders. This is an important finding since SNAP23 may be essential for GLUT4 translocation in muscle cells and may intracellularly relocate to lipid droplets when lipid accumulates and thereby promote insulin resistance. In the present study HIIT reduced SNAP23, but improved insulin sensitivity was observed. With our current knowledge of the role and function of SNAP23, this is not easy to explain, but it may be a result of greater SNAP23 relocation to GLUT4 and thereby improved GLUT4 translocation. Further investigations are required to elucidate this.

Prior studies report inconsistent results on the effect of HIIT on fasting plasma glucose and insulin concentrations. The present study demonstrates that HIIT does not affect beta-cell function in older subjects without type 2 diabetes as there were no changes in fasting values of plasma glucose or insulin, and the insulin secretion rate and glucose excursion during the OGGT and IVGTT remained unchanged.

Studies have reported that reduced insulin sensitivity in older subjects is due to obesity rather than aging itself, and visceral fat has been shown to be negatively associated with insulin sensitivity. In the present study we found reductions in visceral fat and percent fat which agrees with prior HIIT studies. Visceral fat did correlate negatively with whole-body insulin sensitivity before and after HIIT, but the changes in these two parameters did not correlate, which implies that they are probably coupled indirectly.

Plasma total cholesterol and LDL concentrations were significantly reduced after HIIT, which is applicable to metabolic health. We found no change in plasma FFA or triglycerides, while HDL decreased slightly, but only in females. Other studies have found a reduction in LDL concentration or no change in either of these lipid parameters after 12 weeks HIIT in young or older subjects. The
contrasting findings with the present study may be explained by the fact that subjects in the present study had a less healthy lipid profile at baseline compared to the two other studies.

A strength of the present study was that the duration of only six weeks was sufficient to induce significant changes in insulin sensitivity, glucose metabolism, body composition, plasma lipids and VO$_2$max.

Yet another strength was that the subjects did not alter their diet or physical activity level throughout the intervention and remained weight stable, as planned and expected. The improvements reported are thus most likely caused by HIIT and not by weight changes.

We observed an improved maximal load in the post VO$_2$max test, but we found no changes in lean body mass or estimated quadriceps muscle mass to explain this. We speculate that the time span of the intervention was too short though to increase muscle mass, taking into account that aging may slow down the process of muscle hypertrophy.

**Conclusion**

We demonstrate that HIIT improves metabolic health in older subjects by improving whole-body insulin sensitivity, body fat%, visceral fat mass and plasma lipid profile. Furthermore, no adverse effects of HIIT was observed in this cohort, and we therefore suggest that HIIT can be applied as a time efficient strategy to meet or delay the onset of age induced decline in metabolic health.

**Materials and methods**

**Subjects**

Subjects were recruited through advertisements in local newspapers and met the following criteria for inclusion: 55-75 years, BMI > 27 kg/m$^2$, Caucasian, sedentary (International Physical Activity Questionnaire (IPAQ): < 600 MET min/week) and non-smoker. Patients diagnosed with Type 1 or 2 diabetes or other metabolic or heart diseases were excluded from participation. 31 females and 24
males were invited for a screening visit where they completed a health related questionnaire and an IPAQ and had an electrocardiogram performed. Potential subjects were excluded based on physical activity level (IPAQ), medication, disease status, physical limitations, alcohol intake, smoking, lack of time or motivation. 15 females and 13 males were finally included in the study. All subjects gave informed written consent prior to their participation in the study. The study was approved by the Ethical Committee of Copenhagen (journal no. H-3-2012-024) and complied with the Danish Data Protection Agency and the guidelines of the Helsinki Declaration.

During the six weeks HIT training 4 females and 2 males dropped out due to disease and family issues not related to the intervention. In total 11 females and 11 males completed the study of which it appeared 1 female and 1 male had undiagnosed pre-diabetes (HbA1c (%) = 6.1/6.3) and 1 male undiagnosed type 2 diabetes (HbA1c (%) = 6.6) (only measured once).

Experimental design
During the first two weeks of the study each subject went through three test days (test day 1-3), all separated by at least 48 hours (figure 1) (see details below). Test day 1 and 2 were completed in random order. On test day 1, beta-cell function was assessed by an intravenous glucose tolerance test (IVGTT) followed by a hyperinsulinaemic-euglycaemic clamp to assess whole-body insulin sensitivity. On test day 2, blood pressure was measured after 10 minutes rest in sitting position, and height and weight were measured. After a basal blood sample, a muscle biopsy from muscle vastus lateralis was obtained and a 3 hour oral glucose tolerance test (OGTT) was performed. After the OGTT, a light meal was consumed and 1 hour after the OGTT the subjects had maximal oxygen uptake (VO$_2$max) measured. At test day 3, body composition was measured by a dual energy X-ray absorptiometry scan (DXA) (Lunar iDXA, GE Healthcare, Madison, WI, USA) followed by a second VO$_2$max test. Three-four days after test day 3, six weeks HIIT training was initiated, as described below. Test day 4 was initiated 72 hours after the 18th HIIT session by a DXA scan followed by tests similar to test day 2. To maintain the training effect, two HIIT sessions (19 and 20) were performed.
after test day 4. Test day 5 was carried out 48-72 hours after the 20th HIIT session and comprised of an IVGTT and a clamp similar to test day 1. Finally, test day 6 was placed 48-72 hours after test day 5 where a VO$_2$max test was performed.

The subjects were informed not to perform vigorous exercise 24 hours before each visit, and they attended the lab after an overnight fast. The subjects were further asked to maintain their habitual level of physical activity and dietary intake throughout the study, and to weigh and register their nutritional intake for two separate periods of 4 days before and half way through the HIIT intervention.

**HIIT intervention**

During the six week intervention the subjects performed supervised HIIT training on a bicycle ergometer three times a week (session 1-18). To determine the individual training load for the training sessions, the first HIIT session comprised up to 9 intervals of 1 minute with 1 ½ minutes break. The first interval was performed at 85% of the maximal load measured at the VO$_2$max test increasing 10% at each interval. The load in the final interval completed was applied to HIIT session 2-6 and was 177 ± 12 and 253 ± 13 Watt for females and males, respectively. Each HIIT session consisted of 2 minutes warm up at 50 Watts followed by 5 HIIT intervals of 1 minute at the individually determined workload at a cadence of > 50 rounds per minute (RPM). The intervals were interspersed by 1½ minute light cycling at 25 Watts or just resting on the bike. The number of intervals was increased from three to five from session 2 to 4 which was maintained throughout the intervention. At session 6, 12 and 18 the oxygen uptake was measured during the training sessions to confirm that the subjects did train at a high intensity. Heart rate was monitored using a heart rate monitor (Polar T31 Transmitter, Finland) during all sessions.
Tests

**OGTT**
A catheter was placed in a dorsal vein of the hand which was positioned in a heating pad for 10 minutes to arterialize the blood prior to sampling. A basal blood sample was drawn and 75 g glucose dissolved in 300 ml tap water was ingested within 2 minutes. Blood samples were drawn every 10 minutes during the first 60 minutes and then every 30 minutes time 60-180 minutes.

**IVGTT**
A catheter was placed in a cubital vein for injection of glucose (25 g glucose dissolved in 137 ml isotonic saline administered over 1 minute), and in a dorsal vein of the hand placed in a heating pad for blood sampling. Blood samples were taken at time 2, 3, 4, 5, 6, 8, 10, 12, 14, 19, 25, 30 and 40 minutes.

**Hyperinsulinaemic-euglycaemic clamp**
Immediately after the IVGTT a clamp was performed. The high blood glucose level from the IVGTT was initially lowered by a 2 ml insulin bolus followed by 2 hours constant insulin infusion (80 mU·min⁻¹·m⁻²). The plasma glucose concentration was measured every 5 minutes and kept at 5 mM by a controlled infusion of 20% glucose. The whole-body insulin sensitivity was defined as the glucose infusion rate (GIR) during the final 30 minutes of the clamp when the infusion rate and plasma glucose concentration were in steady state (figure 2).

**Biopsies**
Muscle biopsies were obtained from m. vastus lateralis (Bergström technique). The skin was initially sterilized and Lidocain 5 mg/ml injected to anesthetize the skin and muscle fascia. The muscle tissue was immediately divided in two parts; one was frozen immediately in liquid nitrogen, and the other was mounted in Tissue Tek, frozen in isopentane and transferred to liquid nitrogen. The muscle was stored at -80 °C for later analysis.
**Anthropometry**

Body composition was measured by a dual energy X-ray absorptiometry scan (DXA) (Lunar iDXA, GE Healthcare, Madison, WI, USA). Waist circumference was measured at the midpoint between the top of crista iliaca and the lowest rib. The average of two measurements with a difference < 1 cm was calculated but if the difference was ≥ 1 cm two new measures were made. Thigh volume and mass of muscle quadriceps femoris were estimated as previously described.  

**Maximal oxygen uptake**

The VO₂max was measured twice on two separate test days before the HIIT intervention to allow familiarization with the equipment thus eliminating any learning effects. The highest measured value from the two tests was used as the pre training maximal oxygen uptake of the subject.

The VO₂max test was performed as an incremental test on a bicycle ergometer (Corival LODE cycle, Groningen, The Netherlands). During the test respiratory data were collected by a Cosmed online gas connecting system (Quark PFT Ergo, Cosmed, Rome, Italy). Subjects initially performed 5 minutes warm up at 50 Watt followed by 1 Watt increase every third second until exhaustion. The test was considered valid if a plateau in oxygen consumption was observed, a respiratory exchange ratio (RER) ≥ 1.15 and/or inability to keep the cadence above 50 rounds per minute (RPM).

**Analyses**

**Biochemistry**

Blood samples obtained during the OGTT, IVGTT and hyperinsulinaemic-euglycaemic clamp for analysis of plasma insulin, C-peptide, glycerol, free fatty acids (FFA), triglyceride, cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and high-sensitivity C-reactive protein (hsCRP) were collected into chilled tubes containing heparin (Cat. 367374, BD Albertslund, Denmark), and samples for plasma glucose analysis in fluoride vacutainers (Cat. 368520).
samples were centrifuged for 1 minute at 1200 g at room temperature (RT) and the remaining samples were centrifuged at 2000 g for 10 minutes at 4°C. After centrifugation the plasma was collected and all samples stored at -80°C for later analysis.

Concentration of plasma glucose, FFA, glycerol, cholesterol, hsCRP and triglyceride were measured on a Hitachi Cobas 6000 chemistry analyser (Roche A/S, Hvidovre, Denmark). Insulin and C-peptide were assessed using commercial Elisa Kits (ALPCO Diagnostics, Salem, HN, USA. Insulin: cat. No. 80-INSHU-E01; C-peptide: cat. No. 80-CPTHU-E01.1, E10). Samples were analysed on a Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Slangerup, Denmark).

Analysis of glycated haemoglobin (HbA1c) was performed using a DCA Vantage Analyser (Tarrytown NY, USA).

**Western blot**

Muscle biopsies were freeze dried for 48 hours at -40°C and pressure < 0.5 mBar. Before dissection the biopsies equilibrated 1 hour to RT at maintained pressure. Muscle tissue (4-4.5 mg, dry weight) was dissected and homogenized in 400 µl cold Radio-Immunoprecipitation Assay (RIPA) buffer with a silver bead for 2 minutes using a TissueLyser (Qiagen TissueLyser II Retsch MM400, Hilden, Tyskland). 1 minute centrifugation at 1100 RPM followed by 1 minute ultrasound at 40 kHz (Ultrasonic cleaner, Bronson 200, Roedovre, Denmark).

A Bicinchoninic acid (BCA) assay (Pierce, Rockford, IL, USA) was performed to determine protein concentration in triplicate on a Multiscan FC (Thermo Fisher Scientific Inc. Waltham, MA 02454, USA). A maximal variation coefficient of 5% between triplicates was accepted. Laemmli buffer and MilliQ water were added to obtain equal protein concentration in the samples.

Equivalent amounts of total protein (10-20 µg) from each sample was transferred to two new tubes per sample with one left at RT and the other heated in 95°C for 10 minutes, respectively. The samples, a calibrator and a molecular weight marker (2.5 µl Magicmark, XP western std. & 3 µl High
Range Rainbow molecular weight marker) were loaded on a 26 wells 4-15% Criterion TGX Stain-Free polyacrylamide sodium dodecyl sulphate (SDS) gel (Criterion, Bio-rad, Copenhagen, Denmark), and the proteins were separated during electrophoresis at 100-250 volt. The gels were subsequently activated with UV light for 5 minutes using a LAS 4000 image analyzer (GE Healthcare, Little Chalfont, UK), and a 1 second image was taken. The gels were then transferred to an ethanol activated polyvinylidene fluoride (PVDF) membrane (0.2 µm pores, Bio-Rad, Copenhagen, Denmark) with semi-dry blotting at 25 V in 7 minutes using a Trans-Blot Turbo Transfer System (Bio-Rad, Copenhagen, Denmark) with Trans-Blot Turbo RTA Midi Transfer Packs. A 1 second image was taken with UV light of the membranes with the proteins transferred.

The membranes were blocked in skimmed milk or bovine serum albumin (BSA) diluted in Tris-buffered saline (10 mM Tris Base, 150 mM NaCl, pH 7.4) and 0.05% Tween 20 for 1½ hour at RT. The membranes were incubated over night with a dilution of primary antibody: anti-GLUT4 1:12000 (PA1-1065, Fischer Scientific, Roskilde, Denmark), anti-glycogen synthase 1:4000 (#3893, Cell Signaling, Massachusetts, USA), anti-glycogen phosphorylase 1:12000 (As09 455, Agrisera, Vännäs, Sweden), anti-hexokinase II 1:1000 (ab104836, Abcam, Cambridge, UK) and anti-SNAP23 1:3000 (ab3340, Abcam, Cambridge, UK). Secondary antibodies used were polyclonal goat anti-rabbit horseradish peroxidase conjugated 1:2000 (P0448, DAKO, Glostrup, Denmark). The blots incubated 1 minute with ECL detection reagents (Amersham western blotting detection reagents, GE Healthcare, UK) and visualized with LAS 4000 image analyzer (GE Healthcare, Little Chalfont, UK). Subsequent quantification of the proteins of interest was performed using ImageQuant TL software version 7.0 (GE Healthcare). The intensity of each band of interest was normalized to total protein which was measured by Stain-Free fluorescence (UV picture after transfer). Procedure and normalization of protein have previously been described 42, 48. To compare the samples loaded on different gels all samples were quantified relative to a calibrator (pool of all samples) which was loaded on all gels in minimum three lanes.
Citrate synthase analysis

Citrate synthase (CS) activity was analysed as previously described \(^{42}\).

Statistical analyses

The insulin secretion rate (ISR) was calculated by using the ISEC software program \(^{49}\) by deconvolution of C-peptide concentrations and using population-based estimates of C-peptide kinetics.

Data were analysed by two-way analyses of variance (ANOVA) with repeated measurements to evaluate HIIT induced and gender specific changes as well as interactions between these two factors. Holm-Sidak method was applied as post-hoc test. Data were log transformed if normal distribution or equal variance failed. All analyses were carried out using Sigmaplot 13.0. Statistical significance was considered p<0.05. Data are presented as mean ± SEM.

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Conflict of interest

There is no conflict of interest to declare.


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Table 1

Subject characteristics

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<td>Thigh volume (cm³)</td>
<td>7.46±0.4</td>
<td>8.9±0.5</td>
<td>0.028</td>
<td>NS</td>
</tr>
<tr>
<td>Est. quad. fem. muscle mass (kg)</td>
<td>2.64±0.1</td>
<td>3.10±0.2</td>
<td>0.028</td>
<td>NS</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>43.3±1</td>
<td>59.6±2</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>44.8±2</td>
<td>34.7±2</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Visceral fat (kg)</td>
<td>1.94±0.3</td>
<td>1.86±0.2</td>
<td>NS</td>
<td>0.017</td>
</tr>
<tr>
<td>Leg lean mass (kg)</td>
<td>15.5±0.4</td>
<td>21.0±0.7</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Android fat %</td>
<td>52.9±2</td>
<td>44.9±2</td>
<td>0.015</td>
<td>0.019</td>
</tr>
<tr>
<td>Gynoid fat %</td>
<td>45.0±2</td>
<td>33.2±2</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>FFA (µmol·l⁻¹)</td>
<td>655±54</td>
<td>469±34</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Glycerol (µmol·l⁻¹)</td>
<td>86.9±7</td>
<td>43.4±4</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>hsCRP (mg·l⁻¹)</td>
<td>3.21±1</td>
<td>3.23±1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mmol·l⁻¹)</td>
<td>6.15±0.3</td>
<td>4.94±0.3</td>
<td>0.006</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL (mmol·l⁻¹)</td>
<td>1.87±0.2*</td>
<td>1.35±0.1</td>
<td>&lt;0.009</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mmol·l⁻¹)</td>
<td>4.48±0.3</td>
<td>3.62±0.3</td>
<td>0.037</td>
<td>0.044</td>
</tr>
<tr>
<td>Triglyceride (mmol·l⁻¹)</td>
<td>1.22±0.1</td>
<td>1.35±0.2</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>C-peptide (pmol·l⁻¹)</td>
<td>680±180</td>
<td>566±49</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma insulin fasting OGTT (pmol·l⁻¹)</td>
<td>37.8±7</td>
<td>44.3±7</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma glucose fasting OGTT (mmol·l⁻¹)</td>
<td>4.90±0.2</td>
<td>5.14±0.3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.6±0.1</td>
<td>5.8±0.1</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79.3±2</td>
<td>81.7±4</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115±3</td>
<td>126±6</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Subject characteristics of 22 older overweight and obese males and females performing six weeks high-intensity interval training (HIIT). Interaction between training and gender: *p=0.05, †p<0.001. Visceral fat, C-peptide and HbA1c statistics are based on log10 transformed data. Data are means ± SEM.
Table 2

Glycaemic parameters

<table>
<thead>
<tr>
<th></th>
<th>Females (n=9/11)</th>
<th>Males (n=9/11)</th>
<th>Main effect (p value)</th>
<th>Interaction (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>IVGTT:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose AUC (mM·min⁻¹)</td>
<td>476±23</td>
<td>491±15</td>
<td>510±14</td>
<td>509±18</td>
</tr>
<tr>
<td>Insulin AUC (nM·min⁻¹)</td>
<td>8.89±0.67</td>
<td>8.82±0.76</td>
<td>7.56±1.15</td>
<td>7.82±1.12</td>
</tr>
<tr>
<td>ISR AUC (pmol·min⁻¹·kg⁻¹)</td>
<td>220±20</td>
<td>219±19</td>
<td>142±13</td>
<td>157±15</td>
</tr>
<tr>
<td><strong>OGTT:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose AUC (mM·min⁻¹)</td>
<td>1632±82</td>
<td>1536±67</td>
<td>1577±86</td>
<td>1578±91</td>
</tr>
<tr>
<td>Insulin AUC (nM·min⁻¹)</td>
<td>78.3±11.4</td>
<td>72.6±8.2</td>
<td>67.2±12.8</td>
<td>66.4±13.3</td>
</tr>
<tr>
<td>Glycogen, muscle (nmol·mg dw⁻¹)</td>
<td>327±21</td>
<td>463±29</td>
<td>319±42</td>
<td>499±34</td>
</tr>
</tbody>
</table>

Glycemic parameters in 22 elderly overweight or obese males and females performing six weeks high-intensity interval training (HIIT). IVGTT: n=9 females, 9 males; OGTT and glycogen: n=11 females, 11 males. AUC: Area under the curve, GIR: Glucose infusion rate, IVGTT: Intravenous glucose tolerance test, ISR: Insulin secretion rate, OGTT: Oral glucose tolerance test. Data are means ± SEM.
Table 3

<table>
<thead>
<tr>
<th>Training parameters</th>
<th>Females (n=11)</th>
<th>Males (n=11)</th>
<th>Main effect (p value)</th>
<th>Interaction (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>$\text{VO}_{2} \text{max (ml·min}^{-1}$</td>
<td>$1873\pm46$</td>
<td>$1926\pm82$</td>
<td>$2595\pm137$</td>
<td>$2796\pm175$</td>
</tr>
<tr>
<td>$\text{VO}_{2} \text{max (ml·min}^{-1} \cdot \text{kg}^{-1}$</td>
<td>$23.1\pm1$</td>
<td>$23.9\pm1$</td>
<td>$27.4\pm2$</td>
<td>$29.5\pm2$</td>
</tr>
<tr>
<td>Max work load (watt)</td>
<td>$145\pm5$</td>
<td>$154\pm8$</td>
<td>$198\pm11$</td>
<td>$229\pm12$</td>
</tr>
<tr>
<td>Time to fatigue (sec)</td>
<td>$666\pm25^*$</td>
<td>$715\pm33$</td>
<td>$892\pm42^*$</td>
<td>$1018\pm47$</td>
</tr>
<tr>
<td>CS activity ($\mu$mol·g$^{-1}·\text{min}^{-1}$)</td>
<td>$115\pm14$</td>
<td>$156\pm17$</td>
<td>$128\pm11$</td>
<td>$180\pm12$</td>
</tr>
</tbody>
</table>

Training parameters in 22 older overweight or obese males and females before and after six weeks high-intensity interval training (HIIT). $\text{VO}_{2} \text{max}$: Maximal oxygen uptake, CS: Citrate synthase. Interaction between gender and training: *: p<0.05; ‡: p<0.001. Data are means ± SEM.

Figure 1. Study protocol illustrating test days before and after high-intensity interval training (HIIT). 22 sedentary, overweight or obese males and females, 55-75 years old performed six weeks HIIT. HIIT session 19 and 20 were included to maintain the training effect for between post test days. BP: Blood pressure, clamp: Hyperinsulinaemic-euglycaemic clamp, Diet reg.: Diet registration (4 days), DXA: Dual-energy X-ray absorptiometry, IVGTT: Intravenous glucose tolerance test, OGTT: Oral glucose tolerance test, $\text{VO}_{2} \text{max}$: Maximal oxygen uptake test, Sess.: HIIT session.

Figure 2. Glucose infusion rate (GIR) in steady state (90-120 minute) of hyperinsulinaemic-euglycaemic clamp performed in older, sedentary, overweight or obese males (n=9) and females (n=9) before and after six weeks high-intensity interval training. *p<0.05 main effect. (a) Progression of hyperinsulinaemic-euglycaemic clamp showing main effect in GIR during stable plasma glucose concentration at 90-120 minutes. (b) Grey bars: males, blue bars: females. Data are mean ± SEM.

Figure 3. Pearson correlation between glucose infusion rate (GIR) and visceral fat in 17 elderly, sedentary, overweight and obese males and females performing six weeks high-intensity interval training (HIIT). The correlations presented show the values before (a) and after (b) the intervention. Grey squares: males, blue triangles: females.

Figure 4. Western blot presenting expression of proteins involved in glucose handling in skeletal muscle. Older overweight or obese subjects performed six weeks high-intensity interval training (HIIT). Grey bars: males, blue bars: females. *p<0.05, †p<0.01. Data are means ± SEM.
Figure 2

(a)

- GIR pre
- GIR post
- Glucose pre
- Glucose post

Glucose infusion rate (ml/min·kg)

Time (min)

Glucose (mg/dl)

*
Figure 3

(a)

Visceral fat pre (kg)

GIR pre (mg·min⁻¹·kg⁻¹)

R²=0.70, p=0.002

Females
Males
(b)

![Graph showing the relationship between visceral fat post (kg) and GIR post (mg·min⁻¹·kg⁻¹) for females and males. The correlation coefficient is R=0.69, p=0.002.](image)

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Figure 4

[Diagram showing protein content for various proteins (GLUT4, HK II, GS, GP, SNAP23) across different groups (Male pre, Female pre, Male post, Female post).]