# Decreased insulin-stimulated brown adipose tissue glucose uptake after short-term exercise training in healthy middle aged men.

1 **Decreased insulin-stimulated brown adipose tissue glucose uptake after short-term exercise training in healthy**

2 **middle aged men**

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18 *Short Title:* Exercise training and brown adipose tissue metabolism

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2 **Abstract**

3 Aims: Little is known about the effects of exercise training on brown adipose tissue (BAT) metabolism in humans. We

4 tested the hypothesis that high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT)

5 improve BAT insulin sensitivity.

6 Materials and methods: Healthy middle-aged men (n=18, age 47 [CI: 49, 43] years, BMI 25.3 [CI: 24.1-26.3] kg·m$^{-2}$,

7 VO$_{2peak}$ 34.8[CI: 32.1, 37.4] ml·kg$^{-1}$·min$^{-1}$) were recruited and randomized into six HIIT or MICT sessions within two

8 weeks. Insulin-stimulated glucose uptake was measured using [$^{18}$F]FDG-PET in BAT, skeletal muscle, and abdominal

9 and femoral subcutaneous and visceral white adipose tissue depots before and after the training interventions.

10 Results: Training improved VO$_{2peak}$ (*P*=0.0005), insulin-stimulated glucose uptake into quadriceps femoris muscle

11 (*P*=0.0009) and femoral subcutaneous white adipose tissue (*P*=0.02), but not in BAT, with no difference between the

12 training modes. Using pre-intervention BAT glucose uptake, we next stratified subjects into high BAT

13 (>2.9μmol/100g/min; n=6) or low BAT (<2.9 μmol/100g/min; n=12) groups. Interestingly, training decreased insulin-

14 stimulated BAT glucose uptake in the high BAT group (4.0[2.8, 5.5] vs. 2.5[1.7, 3.6]) (training*BAT, *P*=0.02), whereas

15 there was no effect of training in the low BAT group (1.5[1.2, 1.9] vs. 1.6[1.2, 2.0] μmol·100g·min$^{-1}$). High BAT

16 subjects had lower levels of inflammatory markers compared to low BAT subjects.

17 Conclusions: Subjects with functionally active BAT have an improved metabolic profile compared to subjects with

18 low BAT activity. Short-term exercise training decreases insulin-stimulated BAT glucose uptake in subjects with active

19 BAT, suggesting that training does not work as a potent stimulus for BAT activation.

20

21 **Keywords***: Brown adipose tissue, exercise training, glucose uptake, free fatty acid uptake, positron emission

22 tomography

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5   1   **Introduction**
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8   2   Brown adipose tissue (BAT) has an exceptional thermogenic potential due to mitochondrial uncoupling protein 1
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10  3   (UCP1) [1,2]. BAT is suggested to regulate energy balance in humans, and enhance glucose homeostasis and insulin
11  4   sensitivity, and thus may be a potential therapeutic target for obesity and insulin resistance [3,4]. BAT is activated by
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13  5   cold exposure, and other stimuli, including insulin [5,6]. The effects of exercise training on BAT activity in humans are
14  6   incompletely understood.
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17  7   It is well known that regular exercise improves skeletal muscle insulin sensitivity and whole body homeostasis. We
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19  8   have shown that both high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT)
20  9   improve both whole-body and skeletal muscle insulin-stimulated glucose uptake [7]. Limited studies have
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22  10  investigated the effects of exercise on BAT glucose uptake in humans, and the data, thus far, have not been
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24  11  consistent. One study showed higher BAT glucose uptake in cancer patients who had higher self-reported physical
25  12  activity compared to sedentary subjects [8]. Another study showed significantly lower cold-induced BAT glucose
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27  13  uptake in trained athletes compared to sedentary men [9]. However, both of these studies were cross-sectional
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29  14  investigations, and studies directly determining the effects of exercise training on BAT glucose uptake are lacking. In
30  15  addition, while there has been considerable investigation into the effects of exercise training on glucose metabolism
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32  16  in human subjects, data on the effects of exercise training on fatty acid metabolism in white adipose tissue (WAT)
33  17  and BAT have been much more limited.
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36  18  The aim of this study was to determine if exercise training alters insulin-stimulated glucose uptake in BAT. In
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38  19  addition, we determined if the intensity and duration of exercise training affected BAT metabolism. Sedentary
39  20  middle-aged men (n=18) completed two weeks of HIIT or MICT training. Pre- and post-training insulin-stimulated
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41  21  glucose uptake (GU) and fasting free fatty acid uptake (FFAU) were determined in BAT, white adipose tissue depots
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43  22  and skeletal muscle using 2-[$^{18}$F]flouro-2-deoxy-D-glucose (FDG) and 14(R,S)-[$^{18}$F]fluoro-6-thia-heptadecanoic acid
44  23  (FTHA) and PET/CT. We hypothesized that HIIT exercise training activates BAT, resulting in enhanced insulin-
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46  24  stimulated glucose uptake, and that MICT exercise training increases BAT activity to a lesser extent. Furthermore, we
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48  25  investigated whether BAT activity was related to metabolic profile.
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## 1 Subjects and methods

2 Twenty-eight healthy middle-aged sedentary men (age 40–55 years, BMI 18.5–30 kg/m$^2$, VO$_{2Peak}$-40 ml·kg$^{-1}$·min$^{-1}$)

3 were randomized into two groups: high intensity-interval training (HIIT, n=14) or moderate-intensity continuous

4 training (MICT, n=14). The study was approved by the local ethical committee of the hospital district of South-

5 Western Finland and was carried out in compliance with the Declaration of Helsinki. Informed consent was obtained

6 before any measurements were performed. The recruitment process, and inclusion and exclusion criteria were

7 described previously [10]. In seven subjects supraclavicular PET scanning was not possible due to technical

8 difficulties. During the training intervention, two subjects withdrew from the HIIT group; one due to training-induced

9 hip pain, and another due to illness. One participant from the MICT group withdrew due to personal reasons (total

10 n=18; HIIT, n=7 and MICT, n=11) (Fig 1A and B). This study is part of a larger study titled "The effects of short-term

11 high intensity interval training on tissue glucose and fat metabolism in healthy subjects and in patients with type 2

12 diabetes" (Clinicaltrials.gov #NCT01344928).

## 13 Study design

14 At the initial screening visit, physical examination, ECG, blood sampling, a fasting 2h 75g oral glucose tolerance test

15 (OGTT), and peak oxygen uptake (VO$_{2peak}$) were performed. On the first study day, magnetic resonance imaging (MRI)

16 and fasting [18F]FTHA PET/CT scanning were performed, and on the second day we performed [18F]FDG-PET/CT

17 scanning during a euglycemic-hyperinsulinemic clamp. These studies were repeated after the two-week exercise

18 intervention starting with the [18F]-FTHA-PET study at ~48h, followed by a [18F]FDG-PET study ~72h and OGTT and

19 VO$_{2peak}$ test ~96h after the last training session.

## 20 PET studies

21 The PET/CT images were acquired using GE Discovery TM ST System (General Electric Medical Systems, Milwaukee,

22 WI, USA). The participants fasted ≥12 hours before the PET studies, avoided physical activity and both caffeinated

23 and alcoholic drinks 48 hours before the PET scans. More detailed information is provided in the Supplemental

24 Methods.

## 25 Euglycemic hyperinsulinemic clamp, exercise intervention, peak oxygen uptake test and indirect calorimetry

26 The euglycemic hyperinsulinemic clamp technique was used as described previously [7]. The FDG-PET study was

27 performed when the subject reached the stable glucose concentrations at the level of 5 mmol/l (within 5 % range for

28 at least 15 min) after positioning into the PET scanner. Whole body insulin-stimulated glucose uptake rate (M-value)

29 was calculated from the measured glucose values collected at steady state during the PET scan. Participants were

30 randomized into HIIT and MICT exercise intervention protocols. These protocols consisted of six training sessions

31 over a period of two weeks. Sessions 1 and 2 consisted of 4 x 30s of all-out cycling bouts with 4 min of recovery

1  between the bouts (Monark Ergomedic 828E, Monark, Vansbro, Sweden). Sessions 3 and 4 consisted of 5 bout and

2  sessions 5 and 6 consisted of 6 bouts.  Each bout started with 5-s acceleration to maximal cadence without any

3  resistance, followed by an immediate increase of the load (7.5% of whole body weight in kg) for 30s. A session of

4  MICT consisted of cycling at an intensity of 60% of $VO_{2peak}$ (Tunturi E85, Tunturi Fitness, Almere, The Netherlands).

5  The duration of cycling was 40 mins for session 1 and 2, 50min of session 3 and 4, and 60min for sessions 5 and 6

6  [10]. Peak oxygen uptake ($VO_2$Peak) was determined a week before the exercise intervention started and ~96 hours

7  after the last exercise session, as described previously [7].  The open-system indirect calorimetry (Deltatrac®) was

8  used for the measurement of $O_2$ consumption ($VO_2$) and $CO_2$ production ($VCO_2$) to calculate whole-body energy

9  expenditure and substrate oxidation rates [11,12]

10  **BAT mass calculation**

11  BAT mass was analyzed by thresholding the voxels from all the potential sites of BAT (cervical, supraclavicular, and

12  axillary adipose depots) based on Hounsfield units (HU) in CT image (-250 and -50 HU). All voxels above 2.9

13  µmol/100g/min glucose uptake on parametric PET images were included. Lastly, the volume of all these voxels ($cm^3$)

14  was converted into mass by using BAT density of 0.92 $g/cm^3$ [6,13,14].

15  **Body composition**

16  MRI scans were done using Philips Gyroscan Intera 1.5 T CV Nova Dual scanner (Philips, Amsterdam, Netherlands).

17  Abdominal area axial T1 weighted dual fast field echo images (TE 2.3 and 4.7 ms, TR 120ms, slice thickness 10mm

18  without gap) were obtained. Abdominal subcutaneous and visceral adipose tissue masses were analyzed using

19  SliceOmatic software v.4.3 (http://www.tomovision.com/products/sliceomatic.htm). To obtain the tissue mass, the

20  pixel surface area was multiplied by the slice thickness and the density of adipose tissue 0.9196 kg/l [15].

21  Bioimpedance monitor (InBody, 720, Mega Electronics, Kuopio, Finland) was used to measure body fat percentage.

22  **Other measurements**

23  Serum adipokine concentration of NGF, IL-6, IL-8, Leptin, HGF, MCP-1, and TNF- α were analyzed using the Adipokine

24  Magnetic Bead Panel 2 (Cat#HADK2MAG-61K, Millipore, Billerica, MA) on the Luminex-Multiplex analyzer (Millipore,

25  Billerica,MA). Plasma catecholamines were analyzed using a chromsystems reagent kit for HPLC analysis

26  (Chromsystems Instruments and Chemicals GmbH, Munich, Germany) with the Agilent ChemStation

27  chromatography program. Plasma total and HDL-cholesterol, triglycerides, and glucose were measured from the

28  venous blood samples with an automatized enzymatic assay and insulin using automatized electro-

29  chemiluminescence immunoassay (Cobas 8000, Roche Diagnostics GmbH, Mannheim, Germany). LDL-cholesterol

30  concentration was calculated using the Friedewald formula.

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1 **Statistical methods**

2 Descriptive statistics are presented by model-based means and 95% confidence intervals (CI). Normal distribution of

3 the variables was assessed using the Shapiro-Wilk test, and logarithmic transformation was done for non-normally

4 distributed values (glucose uptake data, muscle and VIS FFAU, BAT mass, insulin levels, HDL, IL-6, TNFα, whole body

5 resting energy expenditure, and catecholamines). Statistical analyses were performed with hierarchical linear mixed

6 models compound symmetry covariance structure, including one within-factor (training; before and after

7 intervention in whole group) interaction term (training*group; the HIIT and MICT groups behaved differently for the

8 change in parameter with significant differences between the training modes) and (training*BAT; the high BAT and

9 low BAT groups behaved differently for the change in parameter with significant difference between them). Missing

10 data points were accounted for by restricted maximum likelihood estimation within the linear mixed models.

11 Correlation analyses were performed between the variables on whole group level (n=18) using Pearson's correlation.

12 All values are reported as model-based mean (SAS least squares means) values from all of the parameters measured

13 before and after training. *P*<0.05 was considered statistically significant. The analyses were performed using SAS

14 System, version 9.3 for Windows (SAS Institute Inc., Cary, NC, US).

15

16 **Results**

17 **Effects of HIIT and MICT**

18 There were no differences in anthropometrics and glucose and lipid profiles between HIIT and MICT groups before

19 the intervention (Supplemental Table 1A and B). After training, aerobic capacity increased 6% (training *P*=0.0005),

20 and this tended to be higher after HIIT (9.2%) compared to MICT (3.5%) training (training*group *P*=0.06). HIIT

21 training lowered LDL cholesterol (training *P*=0.0003, training*group *P*=0.01), and tended to lower total cholesterol

22 more than MICT training (training *P*=0.002, training*group *P*=0.06). Both training modes reduced whole-body fat

23 percentage by 4% (training *P*=0.0005) and visceral fat by 8% (training *P*=0.009).

24 Training improved insulin-stimulated glucose uptake in the quadriceps femoris muscle (training *P*=0.0009) and in

25 femoral white adipose tissue (WAT), but training effect was not significantly different between the groups (training

26 *P*=0.02) (Supplemental Fig 1A and B). Training did not change the insulin-stimulated glucose uptake rate in BAT,

27 abdominal subcutaneous WAT, or in visceral adipose tissue (Supplemental Fig 1B). Exercise training or training mode

28 did not alter FFAU in muscle or BAT (Supplemental Fig 1C and D). FFAU tended to decrease in visceral adipose tissue

29 (training *P*=0.07, training*group *P*=0.11) and in abdominal subcutaneous WAT, with a trend towards greater

30 decrease after MICT than HIIT (training *P*=0.08, training*group *P*=0.06) (Supplemental Fig 1D).

1   At baseline, BAT glucose uptake correlated inversely with BMI (r=-0.55, *P*=0.02), body weight (r=-0.61, *P*=0.01), and

2   waist circumference (r=-0.52, *P*=0.03), and positively with HDL (r=0.62, *P*=0.01). BAT glucose uptake also correlated

3   with whole-body insulin sensitivity (M-value) (r=0.64, *P*=0.004), quadriceps muscle glucose uptake (r=0.47, *P*=0.04),

4   and visceral fat glucose uptake (r=-0.55 *P*=0.02). A positive correlation was found between BAT FFAU and muscle

5   FFAU (r=0.73, *P*=0.002).

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7   **Subjects with low and high BAT activity**

8   BAT activity is highly variable between individuals and we have previously proposed that glucose uptake >2.9

9   μmol/100g/min represents functionally active BAT during euglycemic hyperinsulinemic clamp [5,6]. Therefore, all

10  subjects were re-stratified into high BAT activity (n=6) and low BAT activity (n=12) groups, based on the baseline

11  insulin-stimulated BAT glucose uptake cut-off value of 2.9 μmol/100g/min (Fig 2A and B) [6]. Compared to low BAT

12  subjects, high BAT subjects had lower body adiposity and leptin concentration, greater whole-body insulin

13  sensitivity, and higher HDL cholesterol at baseline, suggesting a healthier metabolic phenotype (Table 1A). High BAT

14  subjects also showed lower IL-6, but higher circulating MCP-1 levels. During insulin stimulation, oxidation of

15  carbohydrates was significantly higher in high BAT than low BAT group (Table 1B), which is in line with better M-

16  value. Despite the higher BAT glucose uptake, no difference was observed in BAT FFAU (Fig 2D), BAT mass, BAT

17  radiodensity (Table 1A), or quadriceps muscle glucose uptake (Fig 3A) between groups at baseline. In contrast,

18  visceral adipose tissue glucose uptake was significantly higher (Fig 3B), and quadriceps muscle FFAU tended to be

19  higher in the high BAT than low BAT group (*P*=0.06, Fig 3C).

20  **Exercise training changes substrate metabolism in high BAT and low BAT subjects**

21  Two weeks of training decreased insulin-stimulated BAT glucose uptake in high BAT subjects, whereas there was no

22  change in low BAT subjects (training*BAT *P*=0.02, Fig 2B and C). In contrast, training decreased BAT FFAU in the low

23  BAT group, but not in high BAT group (training*BAT *P*=0.01, Fig 2D and E). There were also no changes in BAT mass

24  or radiodensity (Table 1A) before and after training. There was no association between the changes in BAT glucose

25  uptake and changes in $VO_{2peak}$, or between the changes in BAT glucose uptake and M-value.

26  Exercise training resulted in similar increases in quadriceps muscle glucose uptake in both high and low BAT subjects

27  (training *P*=0.0009, training *BAT, *P*=0.25, Fig 3A). In contrast, exercise-induced increases in glucose uptake in

28  femoral WAT tended to be higher in high BAT compared to low BAT subjects (training *P*=0.02, training*BAT *P*=0.07,

29  Fig 3B). Training had no effect on muscle FFAU in either group (Fig 3C). Interestingly, training affected IL-6

30  concentrations differently in high BAT and low BAT groups, with the high BAT group having an increase and  the low

31  BAT group having a decrease in IL-6 (training*BAT *P*=0.04, Table 1A). Epinephrine concentrations increased in both

32  groups after training (training P=0.01, training*BAT *P*=0.53, Table 1B).

1 **Discussion**

2 We investigated the effects of short-term exercise training (HIIT and MICT) on BAT metabolism in sedentary men

3 using FTHA and FDG PET/CT-scanning. Training increased aerobic capacity and muscle insulin sensitivity, but,

4 interestingly, decreased BAT insulin-stimulated glucose uptake in subjects who had highly active BAT to start with.

5 We also showed that subjects with high BAT activity had a better metabolic profile compared to subjects with low

6 BAT activity before training.

7 To our knowledge, this is the first study in which BAT metabolism has been quantitated before and after a controlled

8 exercise training intervention. In contrast to our hypothesis, we found that two weeks of exercise training decreases

9 insulin-stimulated BAT glucose uptake in subjects with high BAT activity before the intervention. Our results are in

10 line with the recent cross-sectional data, showing reduced cold-stimulated BAT glucose uptake in athletes compared

11 to sedentary subjects [9]. Of note, all the subjects in the study by Vosselman et al. seemed to have high BAT activity

12 based on 18-FDG uptake values measured using semi-quantitative standard uptake values (SUVs) [9]. In the present

13 study, instead of SUVs, we used quantitative dynamic PET data and performed an intervention study for sedentary

14 subjects [16]. While most human BAT glucose uptake studies use cold exposure, we studied BAT glucose uptake

15 during hyperinsulinemia, which has been shown to associate with cold-induced BAT glucose uptake [17]. Insulin

16 stimulation has been shown to increase BAT glucose uptake 5-fold compared to the fasting state in room

17 temperature in subjects with functional BAT [6]. Indeed, it can be speculated that the subjects with high BAT have

18 higher BAT insulin sensitivity.

19 Although the intervention period in the present study was short, it markedly increased the aerobic capacity and the

20 insulin-stimulated muscle glucose uptake (muscle insulin sensitivity). As BAT is an insulin sensitive tissue, we

21 hypothesised that training would also improve the BAT insulin sensitivity (BAT glucose uptake during insulin

22 stimulation). Interestingly, the results showed that training decreased BAT glucose uptake in the group with higher

23 BAT insulin sensitivity at baseline. BAT is likely not a source of energy during exercise; thus, it can be questioned why

24 would the human body reduce BAT substrate uptake after training? We found no change in whole-body energy

25 consumption and only a tendency towards improved whole-body insulin sensitivity. Thus, to maintain whole body

26 homeostasis, one reason for the decreased BAT glucose uptake after the exercise training might be that the body

27 decreases BAT glucose uptake in order to provide substrate to the increased skeletal muscle glucose uptake [18,19].

28 However, when we tested skeletal muscle glucose uptake as a covariate, we found that the change in skeletal muscle

29 glucose uptake did not explain the decrease in BAT glucose uptake.

30 In rodents, we have shown that exercise-trained rats have more glycogen in BAT compared to non-trained animals

31 (Jessen and Goodyear, unpublished data). Thus, if this is also true in humans, the increased glycogen levels post-

32 training may inhibit the insulin-stimulated glucose uptake into BAT explaining the found reduction in BAT glucose

33 uptake after the training in the present study.

1 In rats, 8 weeks of endurance training decreased BAT mass, UCP-1 expression, and BAT thermogenic activity [20] .

2 Thus, it was suggested that the changes in BAT activity after exercise may be due to a decreased need for BAT heat

3 production since, during exercise, working muscles produce excess heat [20]. In the present study, we did not find

4 any correlation between BAT glucose uptake and BAT mass at baseline or a change in BAT mass after two weeks of

5 training intervention.

6 BAT radiodensity is used as a marker of BAT intracellular triglyceride content [21]. Lower BAT radiodensity in

7 overweight and type 2 diabetic subjects compared to healthy lean controls has been suggested to indicate a shift

8 towards BAT lipid storage or a lipolytic dysfunction of BAT [22,23]. Acute cold exposure increases BAT radiodensity

9 indicating a decrease in BAT lipid content [21,24]. We did not find any change in BAT radiodensity suggesting no

10 exercise-induced effect in BAT lipid content.

11 We found that exercise training increased skeletal muscle insulin-stimulated glucose uptake, but not FFAU, which is

12 consistent with previous data [18,25]. Here, we also found that insulin-stimulated glucose uptake increased in

13 femoral subcutaneous adipose tissue, and the improvement tended to be higher in HIIT compared to MICT group.

14 Previously, moderate or high-intensity exercise training has not been found to directly improve insulin-stimulated

15 glucose uptake in the abdominal or femoral subcutaneous adipose tissue. However, adipose tissue GLUT4 protein

16 expression has been shown to increase in type 2 diabetic subjects after 4-weeks bicycle training [26] and previous

17 cross-sectional studies show that endurance athletes have higher abdominal subcutaneous adipose tissue insulin

18 sensitivity than untrained subjects [27]. It is noteworthy that in the present study, the insulin-stimulated glucose

19 uptake increased only in femoral subcutaneous adipose tissue and not in other fat depots. Femoral subcutaneous

20 adipose tissue is not involved in muscle contraction as such, but is located in close vicinity to the exercising muscles.

21 Thus, it is possible that exercising skeletal muscles releases myokines to local adipose tissue depots [19] which may

22 mediate local adipose tissue glucose uptake.

23 Interestingly, after stratifying subjects based on our previously defined cut-off criteria for the BAT activity, we found

24 that high BAT and low BAT subjects showed clear differences in baseline characteristics before the training

25 intervention. The high BAT subjects in the present study had lower body adiposity and visceral fat mass along with

26 markedly higher insulin sensitivity and HDL cholesterol concentration, which all have been shown to associate with

27 BAT glucose uptake [4,28-31]. The high BAT group also had higher whole-body and muscle insulin sensitivity, as well

28 as higher carbohydrate and lower fat oxidation during hyperinsulinemia, indicating improved metabolic flexibility in

29 changing from fat oxidation during fasting to carbohydrate oxidation during hyperinsulinemia. These data support

30 the concept that high baseline BAT activity may protect against adiposity and insulin resistance.

31 High BAT subjects also had lower levels of IL-6 and leptin, possibly due to lower body adiposity and higher levels of

32 monocyte chemoattractant protein 1 (MCP-1).  IL-6 and MCP-1 are secreted from macrophages, among other

33 tissues, and can have both pro- and anti-inflammatory effects. Elevated levels of these cytokines associate with

1  insulin resistance and are seen in obesity and type 2 diabetes in humans [32,33]. In line with previous studies, high

2  BAT subjects had lower levels of leptin compared with low BAT subjects in the present study [34]. Indeed, the

3  present study cannot determine whether high BAT activity contributes to a positive profile of adipokines or the

4  opposite. However, the present study highlights that BAT can be a key player in metabolic regulation and a possible

5  target for pharmacological treatment of insulin resistance.

6  In rodents, BAT has been shown to secrete IL-6, and exercise-induced IL-6 activates subcutaneous adipose tissue

7  beiging [35]. Stanford et al. have shown that BAT transplantation into the visceral cavity in mice increased circulating

8  IL-6 levels, which was accompanied by enhanced energy consumption, reduced adiposity, and improved glucose

9  homeostasis in the recipient wild type but not in IL-6 knock out mice indicating that these effects were IL-6

10  dependent [36]. They hypothesized that the IL-6 dependent effects may lead to the promotion of lipolysis and

11  increased insulin sensitivity in WAT and heart through an increase in GLUT1 protein expression [36]. In contrast to

12  our findings, Vosselman et al. did not find a correlation between IL-6 and cold-induced BAT activity in humans [9].

13  Thus, the role of IL-6 in BAT metabolism and function in humans warrants further investigation.

14  In summary, the present study suggests that humans with active BAT exhibit a metabolically more favourable

15  phenotype compared to those without active BAT. BAT appears to behave differently from skeletal muscle in

16  response to short-term exercise training, decreasing insulin-stimulated BAT glucose uptake. Thus, exercise training

17  may downregulate BAT glucose metabolism in human subjects. The physiological role for the decreased BAT

18  activation with exercise training will be important to understand given the attention to develop novel activators of

19  BAT for increased energy expenditure and weight loss. The number of subjects in our study was small and further

20  investigations are warranted to confirm our findings.

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4    1    **Conflict of Interest**
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6    2    None of the authors had any conflict of interest regarding any aspect of this manuscript.
7
8
9    3    P.M. contributed to analysis, result interpretation and to the writing of the manuscript. V.S., J.J.E., R.J.M., A.M.,
10
11    4    J.C.H., K.A.V. and J.K. contributed to the acquisition of data. K.K. M., J.J.E., E.L., R.J.M., M.U.D. contributed to data
12    5    analysis. P.N., L.J.G., R.J.M., J.J., R.P., J.K. and K.K.K. contributed to the study design and critical revision of the
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14    6    manuscript for important intellectual content. J.C.H. contributed to the study design, writing and drafting the
15
16    7    manuscript. All authors approved the last version of the manuscript. J.C.H. is the guarantor of this work and, as such,
17
18    8    had access to all study data and takes responsibility for the integrity of the data and the data analysis.
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1

2 **Figure legends**

3 Fig 1. (A) Study design for the sub study of the larger HITPET (NCT01344928) study project including 18 healthy

4 middle-aged men randomized into two training modes, high intensity interval training (HIIT) and moderate intensity

5 continuous training (MICT). (B) Study design showing the division of subjects into high BAT and low BAT groups.

6 Fig 2. (A) Division of subjects in high BAT (circles) and low BAT groups (triangles) with a cutoff point of (2.9

7 μmol/100g/min) at baseline. (B) BAT insulin stimulated glucose uptake (GU) before (white bars) and after the

8 exercise intervention (grey bars). (C) Insulin stimulated BAT glucose uptake in each individual in high BAT group

9 before and after the exercise intervention. (D) fasting free fatty acid uptake (FFAU) in high BAT and low BAT groups

10 before and after the exercise intervention.  (E) Fasting free fatty acid uptake in each individual in low BAT group

11 before and after the exercise intervention. ##$P$<0.0001 #$P$ = 0.03 difference between the groups at baseline,

12 ††$P$=0.01, †$P$=0.02 indicates whether high BAT and low BAT groups behaved differently for the change in parameter

13 with significant difference between them (training*BAT).

14

15 Fig 3. Differences between high BAT and low BAT groups in insulin stimulated glucose uptake (GU) (A and B) and

16 fasting free fatty acid uptake rates (FFAU) (C and D) in muscle and different adipose tissue depots pre and post

17 training. QF, quadriceps femoris muscle; BAT, brown adipose tissue; WAT (A), abdominal subcutaneous white

18 adipose tissue; WAT (F), femoral subcutaneous white adipose tissue; VIS, abdominal visceral adipose tissue. Data are

19 means and (95% CI). #$P$<0.0001 differences between the groups at baseline, *$P$=0.02, **$P$=0.0009 mean changes in

20 pre and post measurements in whole group (training), †$P$=0.02, ††$P$=0.01 indicates whether high BAT and low BAT

21 groups behaved differently for the change in parameter with significant difference between them (training*BAT).

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2  Table 1A. Characteristics of high BAT and low BAT subjects.
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| | High BAT (n=6) | | Low BAT (n=12) | | P values | |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Training | Training * BAT |
| **Anthropometrics** | | | | | | |
| **BMI(kg·m$^2$)** | 23.6(22,25)$^{\#}$ | 23.7(22,26)$^{\#}$ | 26.9 (26,28) | 26.8 (26,28) | 0.93 | 0.75 |
| **Fat percentage (%)** | 18.7 (15, 23)$^{\#}$ | 17.6 (14, 22) | 24.1 (21, 26) | 23.7 (21, 26) | **0.04** | 0.37 |
| **Waist (cm)** | 87.1 (81, 93)$^{\#}$ | 86.3 (80, 93)$^{\#}$ | 98.7 (94, 103) | 98.1 (94,102) | 0.08 | 0.68 |
| **VO$_{2peak}$ (ml/kg/min)** | 36.1(32,41) | 39.1(35,44) | 33.4 (31,36) | 34.9 (32,38) | **0.0005** | 0.16 |
| **Systolic BP (mm Hg)** | 120 (113, 127) | 122 (116,129)$^{\#}$ | 127 (122, 133) | 131 (127,135) | 0.13 | 0.61 |
| **Diastolic BP (mm Hg)** | 77 (71, 83) | 76 (69,81) | 77 (73,81) | 78 (74, 82) | 0.80 | 0.53 |
| **Subcutaneous fat mass (kg)** | 3.3 (1.9, 4.6)$^{(\#)}$ | 3.2 (1.9, 4.5) | 4.8 (3.9, 5.7) | 4.7 (3.8, 5.6) | 0.15 | 0.85 |
| **Visceral fat mass (kg)** | $^{\&}$1.2 (0.7, 2.1)$^{\#}$ | 1.1 (0.6, 1.1) | 3.0 (2.0, 4.3) | 2.8 (1.9 ,4.1) | **0.009** | 0.30 |
| **Glucose Profile** | | | | | | |
| **M value** | 48.9 (36, 62)$^{\#}$ | 49.3 (36, 62) | 31.1 (22, 40) | 36.8 (28, 46) | 0.13 | 0.18 |
| **Glucose$_{fasting}$ (mmol.l$^{-1}$)** | 5.3 (5.1,5.6) | 5.8 (5.4,6.2) | 5.5 (5.3,5.7) | 5.7 (5.5,5.9) | 0.05 | 0.34 |
| **Glucose$_{clamp}$ (mmol.l$^{-1}$)** | 5.1 (4.5,5.7) | 5.3 (4.8,5.8) | 5.5 (5.3,5.8) | 5.5 (5.2,5.7) | 0.47 | 0.35 |
| **Insulin$_{fasting}$ (mmol.l$^{-1}$)** | $^{\&}$3.6 (2.5, 5.4)$^{\#}$ | 3.9 (2.7, 5.8) | 5.7 (4.4,7.4) | 5.7 (4.4,7.5) | 0.54 | 0.62 |
| **Insulin$_{clamp}$ (mmol.l$^{-1}$)** | 79.5 (64,95) | 81.7(66,98) | 72.3 (62,83) | 74.3 (64,85) | 0.59 | 0.99 |
| **HbA1c (mmol·mol$^{-1}$)** | 34.4 (31, 38) | 32.2 (29, 36) | 38.5 (36, 41) | 35.5 (33, 38) | **0.005** | 0.59 |
| **Lipid Profile** | | | | | | |
| **FFA$_{fasting}$** | 0.82(0.5,1.1) | 0.91 (0.6,1.2) | 0.65 (0.4,0.9) | 0.61 (0.4,0.8) | **0.80** | 0.47 |
| **FFA$_{clamp}$** | 0.46 (0.3,0.6) | 0.51 (0.4,0.7) | 0.41 (0.3,0.5) | 0.37 (0.3,0.5) | **0.93** | 0.38 |
| **Cholesterol (mmol·l$^{-1}$)** | 4.85 (4.0, 5.7) | 4.24 (3.4, 5.1) | 5.09 (4.5, 5.7) | 4.61 (4.0, 5.2) | **0.002** | 0.66 |
| **LDL (mmol·l$^{-1}$)** | 2.90 (2.2, 3.6) | 2.46 (1.7, 3.2) | 3.40 (2.9, 3.9) | 3.03 (2.5, 3.5) | **0.004** | 0.77 |
| **HDL (mmol·l$^{-1}$)** | $^{\&}$1.57 (1.4,1.8)$^{\#\#}$ | 1.50 (1.3, 1.7)$^{\#}$ | 1.17 (1.1, 1.3) | 1.12 (1.0, 1.2) | 0.27 | 0.99 |
| **Triglycerides(mmol·l$^{-1}$)** | 0.80 (0.5, 1.1) | 0.59 (0.3, 0.9)$^{\#}$ | 1.10 (0.9, 1.3) | 1.00 (0.8, 1.2) | 0.08 | 0.51 |
| **Others** | | | | | | |
| **Epinephrine** | $^{\&}$0.14 (0.09,0.23) | 0.17 (0.11, 0.27) | 0.14 (0.10, 0.19) | 0.25 (0.18,0.34) | **0.02** | 0.19 |
| **Norepinephrine** | $^{\&}$1.67 (1.2, 2.2) | 2.09 (1.6, 2.8) | 1.95 (1.6, 2.4) | 2.17 (1.8, 2.6) | 0.13 | 0.57 |
| **NGF (pg/ml)** | $^{\&}$0.64 (0.3, 1.5) | 0.50 (0.2, 1.4) | 1.13 (0.7, 1.9) | 1.39 (0.8, 2.4) | 0.95 | 0.37 |
| **IL-6 (pg/ml)** | $^{\&}$0.19 (0.04, 0.9)$^{\#}$ | 0.28 (0.05,1.5) | 1.33(0.5,0.3) | 0.41 (0.2,1.0) | 0.27 | **0.04** |
| **IL-8(pg/ml)** | 4.86 (2.5,7.2) | 4.42 (1.9,6.9) | 6.42(5.1,7.8) | 6.15 (4.8,7.5) | 0.48 | 0.87 |
| **Leptin (pg/ml)** | 1625(1723,432)$^{\#}$ | 1769(1129,4688) | 5030(3499,6610) | 4373(2792,5953) | 0.64 | 0.47 |
| **HGF (pg/ml)** | 348 (128, 568) | 312 (77.9, 547) | 441 (313, 570) | 354 (225, 483) | 0.17 | 0.55 |
| **MCP-1 (pg/ml)** | 285 (204, 366)$^{\#}$ | 300 (217, 384) | 200 (153, 248) | 215 (168, 263) | 0.20 | 0.99 |
| **TNF-α (pg/ml)** | $^{\&}$2.79 (1.7, 4.6) | 2.67 (1.6, 4.5) | 4.99 (3.7, 6.7) | 4.29 (3.2, 5.8) | 0.27 | 0.53 |
| **Bat mass (g)** | $^{\&}$80.1 (43,148) | 81.5 (42,157) | 64.9(43,98) | 61.8 (41,94) | 0.95 | 0.89 |
| **BAT radiodensity (CT HU)** | -87.5 (-93,-82) | -88.4 (-94,-83) | -91.6 (-95,-88) | -91.0 (-95,-87) | 0.93 | 0.58 |

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7  Values are means and 95% confidence intervals. $^{\&}$Log transformation was done to achieve normal distribution.
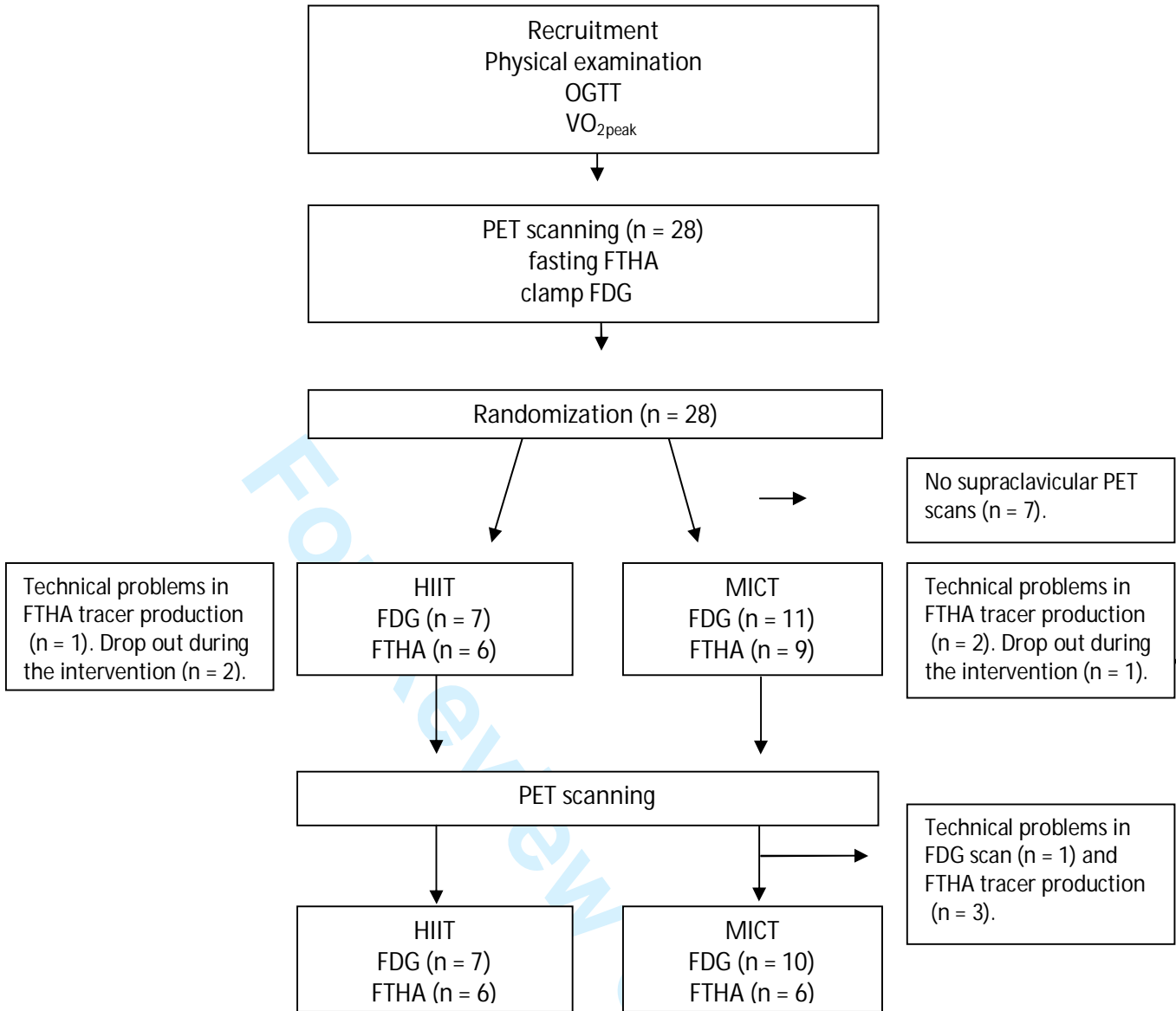8  $^{\#}P<0.05$ and $^{\#\#}P<0.01$ significant difference between groups in corresponding time point. *P* value (training) indicates
9  the the mean changes in pre and post measurements in whole group, *P*-value (training*BAT) indicates whether high
10 BAT and low BAT groups behaved differently for the change in parameter with significant difference between them.

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2  Table 1B. Energy expenditure high BAT and low BAT groups at fast and during hyperinsulinemia.

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| | High BAT (n = 6) | | Low  BAT (n = 12) | | P values | |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Training | Training* BAT |
| **Energy oxidation** | | | | | | |
| **Adj REE$_{fasting}$ (MJ·day$^{-1}$)** | $^{\&}$6.62 (6.1, 7.2) | 6.62 (6.1, 7.2)$^{\#}$ | 6.81 (6.4, 7.2) | 6.86 (6.5, 7.3) | 0.88 | 0.88 |
| **Adj REE$_{clamp}$(MJ·day$^{-1}$)** | $^{\&}$7.09 (6.6, 7.6) | 6.99 (6.5, 7.5)$^{\#}$ | 7.19 (6.8, 7.6) | 7.15 (6.8, 7.5) | 0.58 | 0.85 |
| **Carbohydrate$_{fasting}$(g/min)** | 2.27 (1.6, 3.0) | 1.80 (1.1, 2.5) | 2.18 (1.7, 2.7) | 2.32 (1.8, 2.9) | 0.58 | 0.32 |
| **Carbohydrate$_{clamp}$(g/min)** | 3.66 (2.9, 4.4)$^{\#}$ | 3.08 (2.3, 3.8) | 2.86 (2.3, 3.4) | 2.91 (2.3, 3.4) | 0.34 | 0.26 |
| **Fat$_{fasting}$ (g/min)** | 2.33 (1.6, 3.0) | 2.82 (2.1, 3.5) | 2.65 (2.1, 3.2) | 2.82 (2.0, 3.1) | 0.54 | 0.34 |
| **Fat$_{clamp}$ (g/min)** | 0.98 (0.2, 1.7)$^{\#}$ | 1.51 (0.8, 2.2) | 2.36 (1.8, 2.9) | 2.22 (1.7, 2.7) | 0.47 | 0.27 |
| **Protein$_{fasting}$(g/min)** | 1.13 (1.1, 1.2) | 1.18 (1.1, 1.2) | 1.15 (1.1, 1.2) | 1.14 (1.1, 1.2) | 0.44 | 0.25 |
| **Protein$_{clamp}$ (g/min)** | 1.02 (1.0, 1.1)$^{\#}$ | 1.07 (1.0, 1.1) | 1.11 (1.1, 1.1) | 1.10 [1.1, 1.1) | 0.36 | 0.22 |

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5  Values are means and 95% confidence intervals. $^{\&}$ Log transformation was done to achieve normal distribution. $^{\#}$ P-

6  value <0.05 difference between groups in corresponding time point. *P* value (training) indicates the mean changes in

7  pre and post measurements in whole group, *P*-value (training*BAT) indicates whether high BAT and low BAT groups

8  behaved differently for the change in parameter with significant difference between them.

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Recruitment
Physical examination
OGTT
$VO_{2peak}$

PET scanning (n = 28)
fasting FTHA
clamp FDG

Randomization (n = 28)

No supraclavicular PET scans (n = 7).

Technical problems in FTHA tracer production (n = 1). Drop out during the intervention (n = 2).

HIIT
FDG (n = 7)
FTHA (n = 6)

MICT
FDG (n = 11)
FTHA (n = 9)

Technical problems in FTHA tracer production (n = 2). Drop out during the intervention (n = 1).

PET scanning

Technical problems in FDG scan (n = 1) and FTHA tracer production (n = 3).

HIIT
FDG (n = 7)
FTHA (n = 6)

MICT
FDG (n = 10)
FTHA (n = 6)

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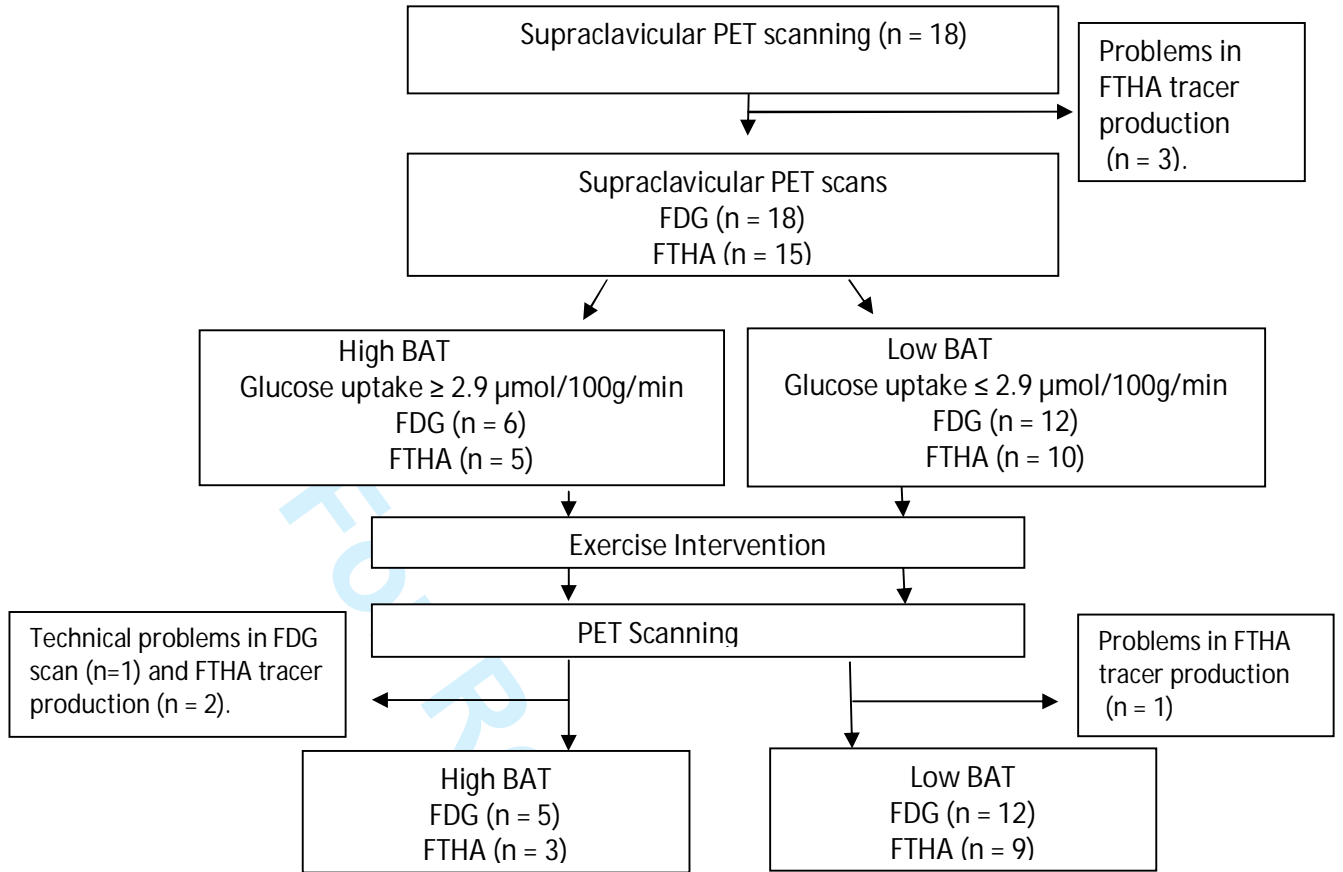41
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                    ┌─────────────────────────────────────────┐   ┌──────────────────┐
                    │  Supraclavicular PET scanning (n = 18)    │──▶│ Problems in      │
                    └─────────────────────────────────────────┘   │ FTHA tracer      │
                                      │                            │ production       │
                                      ▼                            │  (n = 3).        │
                    ┌─────────────────────────────────────────┐   └──────────────────┘
                    │  Supraclavicular PET scans                │
                    │  FDG (n = 18)                             │
                    │  FTHA (n = 15)                            │
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          ┌──────────────────────────┐   ┌──────────────────────────┐
          │ High BAT                 │   │ Low BAT                  │
          │ Glucose uptake ≥ 2.9     │   │ Glucose uptake ≤ 2.9     │
          │ μmol/100g/min            │   │ μmol/100g/min            │
          │ FDG (n = 6)              │   │ FDG (n = 12)             │
          │ FTHA (n = 5)             │   │ FTHA (n = 10)            │
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                    │          Exercise Intervention            │
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  │ Technical problems   │◀─│            PET Scanning                   │─▶│ Problems in FTHA │
  │ in FDG scan (n=1)    │  └─────────────────────────────────────────┘  │ tracer production│
  │ and FTHA tracer      │       │                          │            │  (n = 1)         │
  │ production (n = 2).  │       ▼                          ▼            └──────────────────┘
  └──────────────────────┘  ┌──────────────┐        ┌──────────────┐
                            │ High BAT     │        │ Low BAT      │
                            │ FDG (n = 5)  │        │ FDG (n = 12) │
                            │ FTHA (n = 3) │        │ FTHA (n = 9) │
                            └──────────────┘        └──────────────┘
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2A

BAT GU
(µmol/100g/min)

○ High BAT
△ Low BAT

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2B

BAT GU
(µmol/100g/min)

□ Pre
■ Post

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High BAT    Low BAT

2C

BAT GU
(µmol/100g/min)

○ High BAT

†

Pre    Post

2D

BAT FFAU
(µmol/100g/min)

††

High BAT    Low BAT
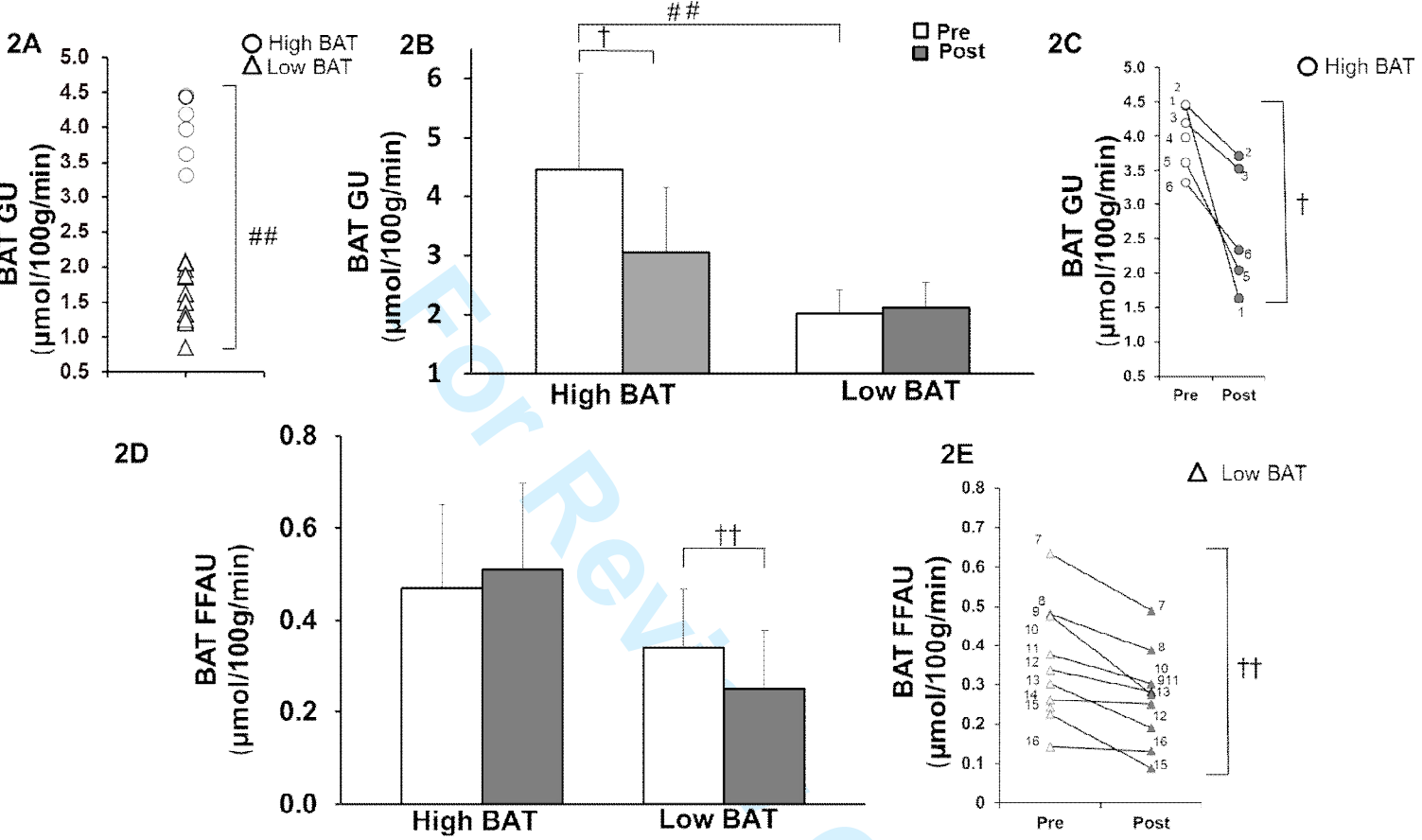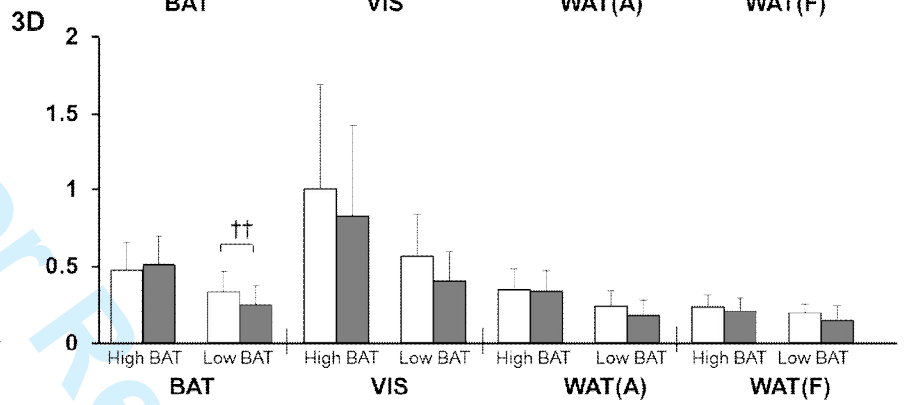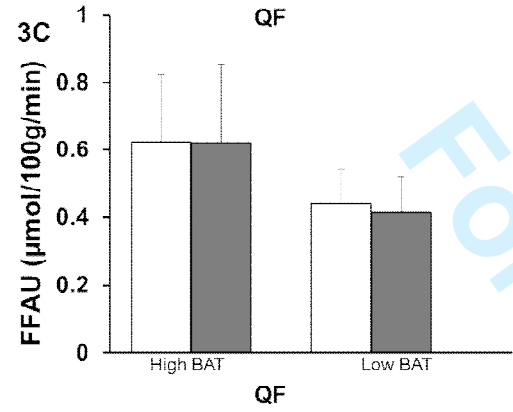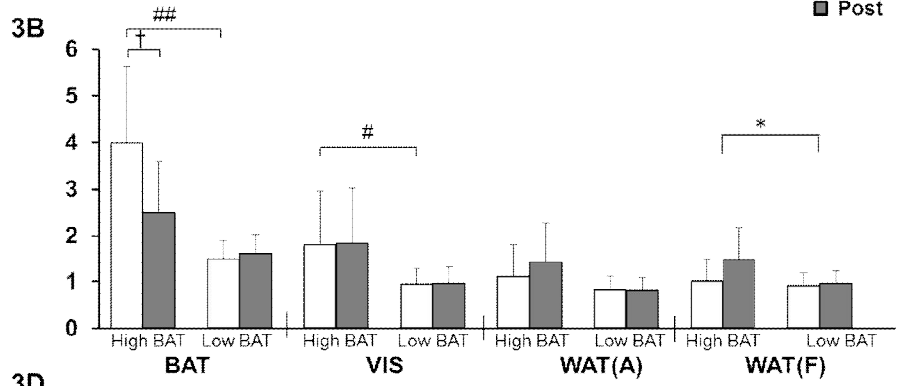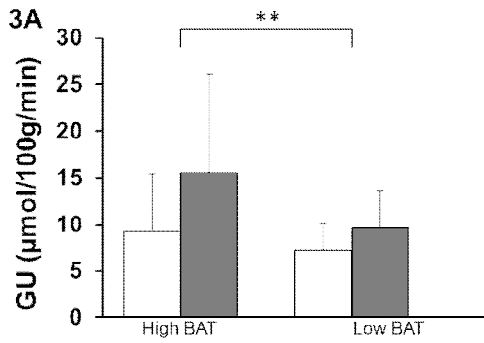
2E

BAT FFAU
(µmol/100g/min)

△ Low BAT

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Pre    Post

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**Supplementary Appendix**

**Supplemental methods**

**PET studies**

The PET/CT images were acquired using GE Discovery TM ST System (General Electric Medical Systems, Milwaukee, WI, USA). The participants fasted ≥12 hours before the PET studies, avoided physical activity and both caffeinated and alcoholic drinks 48 hours before the PET scans. Both antecubital veins were cannulated for the PET studies; one was used for the injection of radiotracers [18F]FTHA and [18F]FDG, and the other one for continuous arterialized blood sampling. The arm that was used for blood sampling was heated with an electrically powered cushion to arterialize the venous blood throughout the scan. Scanning was performed in supine position. On the first day, an [18F]FTHA-bolus (155 [SEM 0.4] MBq) was injected and dynamic imaging of the abdominal region (frames 3x300s) was acquired starting **~**46 minutes after the tracer injection, followed by the femoral (frames 3x300s) and neck (3x300s) regions. On the second PET study day, ~87 minutes after the start of the euglycemic-hyperinsulinemic clamp, [18F]FDG (156 [SEM 0.5] MBq) was injected, and dynamic scanning of the abdominal area started ~48 minutes after the injection. Thereafter, scanning of femoral and neck regions followed as before the intervention. To measure the plasma radioactivity for tracer input function, arterialized venous blood samples were collected repeatedly during [18F]FTHA and [18F]FDG scanning . Plasma radioactivity was measured with an automatic gamma counter (Wizard 1480 3", Wallac, Turku, Finland). Anatomical references were obtained from the acquired CT images.

**Image analysis**

All the data were corrected for dead time, decay, and measured photon attenuation, and then reconstructed by scanner software using 3D-OSEM. Three-dimensional volumes of interest (3D VOIs) were drawn on BAT, abdominal, femoral subcutaneous and visceral adipose tissue, and quadriceps femoris muscle. Tissue time activity curves were obtained from the 3D VOIs, and graphical analysis was used to quantify the fractional uptake rate. Glucose uptake and free fatty acid uptake (FFAU) rates were calculated by multiplying corresponding fractional uptake rates by the mean plasma glucose and FFA level during the imaging period, respectively. For glucose uptake, the value obtained was further divided by lumped constant (LC), a factor used to correct the uptake rate of a PET tracer to that of the substrate of interest. The LC for [18F]-FDG in BAT, WAT, and skeletal muscle was set to 1.14, 1.14, and 1.2 respectively [1,2].

## Reference List

1. Virtanen K.A., Peltoniemi P., Marjamaki P. et al. Human adipose tissue glucose uptake determined using [(18)F]-fluoro-deoxy-glucose ([(18)F]FDG) and PET in combination with microdialysis. *Diabetologia* 2001; **44**:2171-2179.

2. Peltoniemi P., Lonnroth P., Laine H. et al. Lumped constant for [(18)F]fluorodeoxyglucose in skeletal muscles of obese and nonobese humans. *Am J Physiol Endocrinol Metab* 2000; **279**:E1122-E1130.

**Figure Legend**

**Supplemental Fig 1.**

Insulin stimulated glucose uptake (GU) (Fig. 1A and B), fasting free fatty acid uptake (FFAU) (Fig. 1C and D), in different adipose tissue depots before (white bars) and after (grey bars) the training intervention in the high intensity interval training (HIIT) and moderate intensity continuous training (MICT) groups. QF, quadriceps femoris muscle; BAT, brown adipose tissue; WAT(A), abdominal subcutaneous white adipose tissue; WAT(F), femoral subcutaneous white adipose tissue; VIS, visceral adipose tissue. All data is expressed as means and (95% CI). *$P$=0.02, **$P$=0.0009 indicates mean changes in pre and post measurements in whole group (training)

Table 1A. Characteristics of HIIT and MICT groups.

| | HIIT (n=7) | | MICT (n=11) | | *P* values | |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Training | Training * Group |
| **Anthropometrics** | | | | | | |
| **BMI(kg·m$^2$)** | 24.8(23,27)[#] | 23.7(22,26) | 26.9 (26,28) | 26.8(26,28) | 0.93 | 0.75 |
| **Fat percentage (%)** | 21.3 (17, 25) | 20.5 (17, 24) | 21.6 (19, 24) | 20.7 (18, 24) | **0.04** | 0.90 |
| **Waist (cm)** | 91.5 (85, 98) | 90.3 (84, 96) | 94.4 (90, 99) | 94.1 (90, 99) | 0.08 | 0.22 |
| **VO$_{2peak}$ (ml/kg/min)** | 35.0(31,39) | 38.2(34,42) | 34.7 (32,38) | 35.9 (33,39) | **0.0005** | **0.06** |
| **Systolic BP (mm Hg)** | 121 (115, 128) | 123 (117,129) | 125 (121, 130) | 130(125,135) | 0.13 | 0.48 |
| **Diastolic BP (mm Hg)** | 77 (71, 83) | 74 (68,80) | 77 (73,81) | 79 (75, 83) | 0.80 | 0.27 |
| **Subcutaneous fat mass (kg)** | 3.9 (2.6, 5.2) | 3.8 (2.5, 5.1) | 4.0 (3.1, 5.0) | 4.0 (3.0, 4.9) | **0.05** | 0.83 |
| **Visceral fat mass (kg)** | [&]1.9 (1.1, 3.3) | 1.8 (1.0, 3.0) | 1.9 (1.3, 2.9) | 1.7 (1.2, 2.6) | **0.009** | 0.96 |
| **Glucose Profile** | | | | | | |
| **M value** | 44.7 (32, 57) | 49.0 (37, 61) | 35.2 (26, 45) | 37.1 (28, 47) | 0.13 | 0.52 |
| **Glucose$_{fasting}$(mmol·l$^{-1}$)** | 5.5 (5.2, 5.8) | 5.2 (4.7, 5.6) | 5.8 (5.4, 6.1) | 5.7 (5.0, 6.3) | **0.03** | 0.54 |
| **Glucose$_{clamp}$(mmol·l$^{-1}$)** | 5.3 (4.9, 5.8) | 5.4 (5.0, 5.9) | 5.2 (4.8, 5.7) | 5.3 (5.0, 5.7) | 0.47 | 0.89 |
| **Insulin$_{fasting}$ (mmol.l$^{-1}$)** | [&]4.3 (2.9, 6.2) | 4.0 (2.7, 5.8) | 4.9(3.7,6.5) | 5.7(4.3,7.5) | 0.54 | 0.12 |
| **Insulin$_{clamp}$ (mmol.l$^{-1}$)** | 77.5 (63,92) | 77.3 (62,92) | 74.3(63,85) | 78.8(67,91) | 0.59 | 0.55 |
| **HbA1c** | 36.0 (33, 39) | 34.0 (31, 37) | 37.0 (35, 39) | 33.7 (31, 36) | **0.005** | 0.36 |
| **Lipid Profile** | | | | | | |
| **FFA$_{fasting}$** | 0.69(0.4,1.0) | 0.75(0.5,1.0) | 0.78(0.6,1.0) | 0.76(0.5,1.0) | 0.80 | 0.62 |
| **FFA$_{clamp}$** | 0.45 (0.3,0.6) | 0.42(0.3,0.6) | 0.42(0.3,0.5) | 0.46(0.4,0.6) | **0.93** | 0.47 |
| **Cholesterol (mmol·l$^{-1}$)** | 5.30 (4.5, 6.1) | 4.45 (3.6, 5.3) | 4.64 (4.0, 5.3) | 4.39 (3.8, 5.0) | **0.002** | **0.06** |
| **LDL (mmol·l$^{-1}$)** | 3.50 (2.8, 4.2) | 2.78 (2.1, 3.5) | 2.80 (2.3, 3.3) | 2.71 (2.2, 3.2) | **0.004** | **0.02** |
| **HDL (mmol·l$^{-1}$)** | [&]1.35 (1.2, 1.6) | 1.28 (1.1,1.5) | 1.36 (1.2, 1.5) | 1.31 (1.2, 1.5) | 0.27 | 0.83 |
| **Triglycerides (mmol·l$^{-1}$)** | 0.95 (0.7, 1.2) | 0.80 (0.5, 1.1) | 0.95 (0.7, 1.2) | 0.78 (0.6,1. 0) | 0.09 | 0.90 |

Values are means and 95% confidence intervals. [&]Log transformation was done to achieve normal distribution. *P* value (training) the mean changes in pre and post measurements in whole group and *P*-value (training*group) indicates whether groups behaved differently for the change in parameter with significant difference between the training modes.

Table 1B. Indirect calorimetry data of HIIT and MICT

| | HIIT (n = 7) | | MICT (n = 11) | | P values | |
|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | Training | Training* Group |
| **Adj REE$_{fasting}$ (MJ.day$^{-1}$)** | [&]6.72 (6.2, 7.3) | 6.90 (6.4, 7.5) | 6.70 (6.2, 7.1) | 6.58 (6.2, 7.0) | 0.88 | 0.41 |
| **Adj REE$_{clamp}$(MJ.day$^{-1}$)** | [&]7.29 (6.8, 7.8) | 7.22 (6.7, 7.7) | 7.00 (6.6, 7.4) | 6.92 (6.5, 7.3) | 0.58 | 0.95 |
| **Carbohydrate$_{fasting}$(g/min)** | 2.19 (1.4, 2.8) | 1.92 (1.2, 2.6) | 2.27 (1.7, 2.8) | 2.20 (1.7, 2.7) | 0.58 | 0.75 |
| **Carbohydrate$_{clamp}$(g/min)** | 3.53 (2.8, 4.2) | 3.14 (2.4, 3.8) | 2.99 (2.4, 3.5) | 2.85 (2.3, 3.4) | 0.34 | 0.64 |
| **Fat$_{fasting}$ (g/min)** | 2.57 (1.9, 3.2) | 2.68 (1.9, 3.3) | 2.40 (1.8, 2.9) | 2.67 (2.1, 3.1) | 0.53 | 0.79 |
| **Fat$_{clamp}$ (g/min)** | 1.34 (0.6, 2.0) | 1.67 (0.9, 2.3) | 2.00 (1.4, 2.5) | 2.08 (1.5, 2.6) | 0.47 | 0.65 |
| **Protein$_{fasting}$(g/min)** | 1.14 (1.0, 1.1) | 1.16 (1.1, 1.2) | 1.13 (1.0, 1.1) | 1.15 (1.1, 1.1) | 0.44 | 0.94 |
| **Protein$_{clamp}$ (g/min)** | 1.05 (1.0, 1.1) | 1.07 (1.0, 1.1) | 1.09 (1.0, 1.1) | 1.10 (1.0, 1.1) | 0.34 | 0.71 |

Values are means and 95% confidence intervals. [&]Log transformation was done to achieve normal distribution. *P* value (training) indicates the he mean changes in pre and post measurements in whole group and *P*-value (training*group) indicates whether groups behaved differently for the change in parameter with significant difference between the training modes.
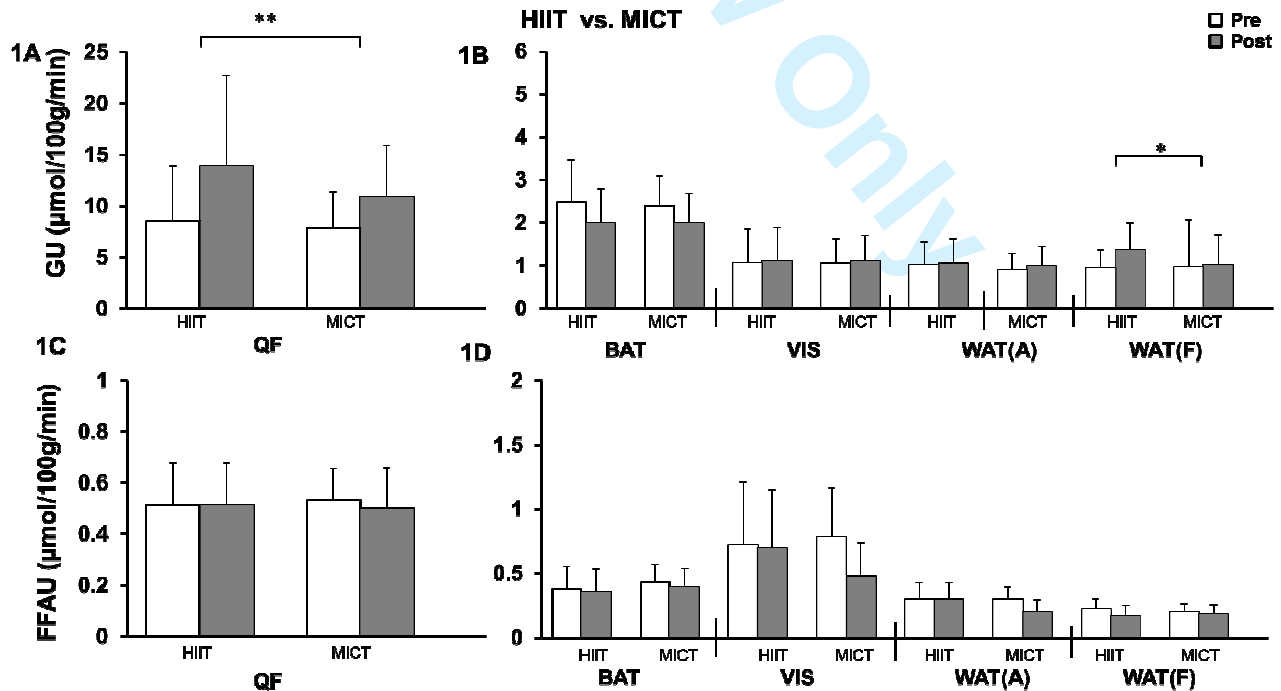
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We thank the reviewers for the feedback regarding our manuscript. We have addressed all the comments made by the reviewers. Please find the point by point answers to the given comments below.

**Referee #1 (Comments to the Author):**

**In the manuscript #DOM-17-0056-OP, the authors investigated whether high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) improve BAT insulin sensitivity.**
**They conclude that subjects with functionally active BAT have an improved metabolic profile, short-term exercise training decreases insulin-stimulated BAT glucose uptake in subjects with active BAT, and that training does not work as a potent stimulus for BAT activation.**

**This study is very interesting as investigations on BAT are required, since it represents a potential therapeutic target for obesity and type 2 diabetes.**

**The manuscript is well-written and the methodology is robust.**

AUTHORS' RESPONSE: We thank the referee for comments and suggestions to improve the clarity and quality of our work. We have now answered to the comments and revised the text accordingly. The specified answers are listed below.

**There is no major comments but the two following suggestions:**

**1) -although the reader as the possibility to found information in the litterature, the HIIT and MICT should be described briefly in the methods section.**

AUTHORS' RESPONSE: We have now described HIIT and MICT in more detailed in the methods section (Page 4, lines 29-31, Page 5, lines 1- 6).

**2) -the authors should acknowledge the limited number of volunteers in this study. Further investigations are still required to confirm the conclusions of the authors.**

AUTHORS' RESPONSE: Done. (Page 10, lines 19-20).

**END.**

**Referee #2 (Comments to the Author):**

The findings by Motiani and co-workers suggests that humans with active BAT exhibit a metabolically favourable phenotype compared to those without active BAT, which has previously been found, also in humans. Those findings, thus confirm previous studies and are not as such novel. However, the finding that insulin-stimulated brown adipose tissue glucose uptake is decreased after short-term exercise training in healthy middle aged men with initially high BAT is interesting and somewhat unexpected. Furthermore, a limited amount studies have investigated the effects of exercise on BAT glucose uptake in humans, and this study is therefore of relevance and some of the findings novel.

However, the manuscript is merely descriptive and there is a complete lack of attempt to investigate the underlying molecular mechanisms. This could be performed if biopsies had been taken. If so, such analysis should be included in the current study.

AUTHORS' RESPONSE: We thank the referee for comments and suggestions to improve the clarity and quality of our work. We have now answered to the comments and revised the text accordingly. The specified answers are listed below.

Comments to be addressed:

1) Although the study is well controlled and data interesting, the complete lack of attempt to elucidate the molecular mechanisms underlying the findings significantly reduces the impact of the study. Were biopsies taken in the current study? And could they be analysed for intracellular signalling and gene expression?

AUTHORS' RESPONSE: We appreciate the comments, but due to the invasive nature of neck biopsies, unfortunately BAT biopsies were not taken in the current study. Despite the lack of signalling studies, we believe the unique physiological data is important for the field.

2) Please include average age of the subjects in the abstract

AUTHORS' RESPONSE: We have now added the average age of the subjects to the abstract (Page 2, line 6).

3) P 7, Line 34-35: "We also showed that subjects with high BAT activity had a better metabolic profile compared to subjects with low BAT activity." Please specify if this was pre-training only or both pre- and post training.

AUTHORS' RESPONSE: We have now tested the group differences after training and added the results in Table 1. We have also edited the text accordingly: "We also showed that subjects with high BAT activity had a better metabolic profile compared to subjects with low BAT activity before training." (Page 8, lines 5-6 and page 10, lines 2-3).

**4) P 7, 48-49: " performed an intervention study for sedentary subjects, excluding the possible effects of genetic factors" how does this exclude genetic factors? Please clarify**

AUTHORS' RESPONSE: This is a good point and we have removed this comment from the manuscript.

**5) Did training increase the amount of BAT?**

AUTHORS' RESPONSE:   We have checked the data and statistics and there is no statistically significant difference between the groups at baseline or in response to training, which may be due to the large variation between the subjects (Page 16, and Table 1A).

**6) P 8, 18-23: "Thus, if this is also true in humans, the increased glycogen levels posttraining may inhibit the insulin-stimulated glucose uptake into BAT explaining the found reduction in BAT glucose uptake after the training in the present study" -Please explain why glycogen would induce insulin resistance in BAT (based on literature) and include reference. Training also increase glycogen in muscle without impairing insulin-stimulated glucose uptake.**

 AUTHORS' RESPONSE: In the discussion, we hypothesize about possibility that increased glycogen in BAT after training may potentially reduce (the need for) glucose uptake in BAT after exercise training. This is a hypothesis, based on presumably different physiological roles of the recovering skeletal muscle and BAT after training. Moreover, glycogen content in skeletal muscle has been suggested to affect insulin-stimulated glucose transport (see: Muscle glycogen content affects insulin-stimulated glucose transport and protein kinase B activity W. Derave, B. F. Hansen, S. Lund, S. Kristiansen, E. A. Richter American Journal of Physiology - Endocrinology and Metabolism Published 1 November 2000 Vol. 279 no. 5, E947-E955).

**7) P8, 34: "BAT radiodensity correlates…" Please specify for non-expert readers what physiological function/mechanism radiodensity is a read-out for.**

AUTHORS' RESPONSE:   We have now described BAT radiodensity more clearly (Page 9, lines 6-10).

Briefly, radiodensity, which is measured using computed tomography, refers to composition of the tissue, in terms of water content. Thus, in BAT, the lower the radiodensity is, the lower is the water content and the higher is the fat content. White adipose tissue has low radiodensity, and the lower BAT radiodensity is, the more it functionally resembles white adipose tissue.

**8) P8 44-45: "The lack of an acute "stress" or activation of sympathetic nervous system at the time of our measurements may explain why there were no changes in BAT radiodensity." -This argument seems untrue given that the authors describe an increase in epinephrine concentrations following both exercise training interventions.**

AUTHORS' RESPONSE:  We agree and have now deleted this from the discussion.

**9) P9, 3-4: "… that exercising skeletal muscles releases myokines to local adipose tissue depots [19] which may mediate local adipose tissue glucose uptake." Other adaptations may occur in adipose tissue in response to exercise training, such as increased GLUT4 content as has previously been reported.**

AUTHORS' RESPONSE:  We appreciate referee's suggestion and have edited the discussion accordingly (Page 9, lines 15-16).

**10) P9, 36-37: "In rodents, BAT has been shown to secrete IL-6, and that exercise-induced IL-6 activates subcutaneous adipose tissue beiging" Remove "that" or restructure sentence.**

AUTHORS' RESPONSE: We have edited the sentence (Page 10, lines 6-7).