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Published in:
American Journal of Physiology: Endocrinology and Metabolism

DOI:
10.1152/ajpendo.00161.2018

Publication date:
2018

Document version
Peer reviewed version

Citation for published version (APA):
High-intensity interval, but not endurance training induces muscle fiber type-specific subsarcolemmal lipid droplet size reduction in type 2 diabetic patients

Han-Chow E. Koh¹, Niels Ørtenblad¹, Kamilla M. Winding³, Ylva Hellsten⁴, Stefan P. Mortensen²,³, Joachim Nielsen¹

¹ Department of Sports Science and Clinical Biomechanics, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
² Department of Cardiovascular and Renal Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
³ The Centre of Inflammation and Metabolism and the Centre for Physical Activity Research, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
⁴ Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Copenhagen, Denmark

Running head: Training effects on subcellular lipid droplet adaptation

Corresponding author:
Stefan P. Mortensen
University of Southern Denmark
Department of Cardiovascular and Renal Research
J.B. Winsløws Vej 21, 3
DK-5000 Odense C, Denmark
Tel: +45 65508324
smortensen@health.sdu.dk

Word count: 249 (Abstract), 5921 (Main text)
Abstract

This study compared the effects of moderate-intensity endurance training and high-intensity interval training on fiber type-specific subcellular volumetric content and morphology of lipid droplets and mitochondria in skeletal muscles of type 2 diabetic patients. Sixteen sedentary type 2 diabetic patients (57±7 years old) were randomized to complete 11 weeks of either 40-min cycling at 50% peak workload (Endurance, n = 8) or 10 1-min cycling intervals at 95% peak workload separated by 1 min of recovery (High-intensity Interval, n = 8), 3 times per week. Assessments for cardiorespiratory fitness, body composition, glycemic control, together with muscle biopsies were performed before and after the intervention. Morphometric analyses of lipid droplets and mitochondria were conducted in the subcellular fractions of biopsied muscle fibers using quantitative electron microscopy. The training intervention increased cardiorespiratory fitness, lowered fat mass and improved non-fasting glycemic control (P < 0.05), with no difference between training modalities. In the subsarcolemmal space, training decreased lipid droplet volume (P = 0.003), and high-intensity interval, but not endurance training, reduced the size of lipid droplets, specifically in type 2 fibers (P < 0.001). No training-induced change in intermyofibrillar lipid droplets was observed in both fiber types. Subsarcolemmal mitochondrial volume was increased by high-intensity interval (P = 0.02), but not endurance training (P = 0.79). Along with improvement in glycemic control, low volume high-intensity interval training is an alternative time-saving training modality that affects subcellular morphology and volumetric content of lipid droplets in skeletal muscle of type 2 diabetic patients.

Keywords: lipid droplets, mitochondria, skeletal muscle, stereology, insulin sensitivity
**Introduction**

Limited adipose tissue expandability and subsequent ectopic fat accumulation leads to lipotoxicity in insulin target tissues and associated complications (reviewed in (6, 46)). Overweight/obese individuals and type 2 diabetic patients are well-documented to exhibit elevated levels of fat around the heart and pancreas and infiltrated fat in the liver and skeletal muscle (inter- and intramyocellular lipids) (reviewed in (32)). Although multiple studies have reported that elevated intramyocellular lipid content is positively associated with skeletal muscle insulin resistance, this proposed relationship is inconclusive as neither has causality been demonstrated (30, 44), nor does it extend to well-trained individuals (21, 35). With no direct link between intramyocellular lipid content per se and insulin sensitivity, subsequent investigations linking levels of lipid intermediates (e.g. diacylglycerols, ceramides) and muscle insulin resistance have produced mixed results (1, 51).

However, recent studies have reported that diacylglycerols and ceramides located at or close to membranes which include the sarcolemma, and not in the cytosolic space, are associated to muscle insulin resistance (4, 11). This indicates that the link with insulin sensitivity depends on specific subcellular localizations of intramyocellular lipids.

Intramyocellular lipids exist as cytosolic lipid droplets (LDs), composed predominantly of a triacylglycerol core with phospholipid monolayer, which have a key role in fatty acid trafficking (19). Under the transmission electron microscope, LDs are observed in two distinct subcellular pools in skeletal muscle fibers – one beneath the sarcolemma (subsarcolemmal) and the other between myofibrils (intermyofibrillar) (42) (see Fig. 1). Our previous study demonstrated that individuals with lower insulin sensitivity, as compared to body mass index (BMI)-matched controls and well-trained athletes, have more lipid droplets in the subsarcolemmal region but not in the intermyofibrillar region of skeletal muscle fibers (42). Furthermore, accumulating evidence indicates that the size of individual LDs could be more closely linked to insulin sensitivity than total intramyocellular lipid content (13, 22, 41). Taken together, these findings underscore the important roles of subcellular localizations and LD size in the relationship between intramyocellular lipids and muscle insulin sensitivity.

It is well-known that type 1 skeletal muscle fibers in general are more oxidative than type 2 fibers, and this may influence the distribution and size of LDs. Indeed, it has been demonstrated that type 1 fibers contain more LDs than type 2 fibers (28, 35, 37) and more recently, the accumulation of LDs
in type 1 fibers was associated with declining insulin sensitivity during acute lipid infusion in humans (10). Moreover, glucose uptake capacity and response of metabolic signaling proteins to exercise were observed to be different between fiber types in humans (2, 29). In addition, muscle tissue is heterogeneous in fiber type distribution which likely has contributed to the diverse results observed from experiments using whole-muscle analyses. Together, these findings warrant studies to take a fiber type-specific approach in investigating lipid metabolism and insulin sensitivity in skeletal muscle in different physiological conditions.

Both acute and long-term adaptations to exercise training are well-established means to improve insulin sensitivity and glycemic control (12), often coinciding with re-partitioning of intramyocellular lipids and mitochondrial function enhancement (16, 17). However, as most of the exercise training studies have used moderate-intensity endurance training (END) which recruits predominantly type 1 fibers, high-intensity interval training (HIIT), involving a higher exercise intensity, will recruit type 2 fibers in addition to type 1 fibers (56, 57), and thereby engage a larger portion of the whole muscle. This may be particularly important in individuals with type 2 diabetes since they have been observed to possess a greater proportion of type 2 fibers than the non-diabetic population (2, 43). Furthermore, HIIT has been demonstrated to improve cardiorespiratory fitness and lower adipose mass across different populations (reviewed in (60)), elevate mitochondrial content (36, 62) and improve insulin sensitivity (52). However, very little is known about the effects of HIIT on subcellular distribution of LDs.

This study aimed to compare the effects of END and HIIT on fiber type-specific subcellular distribution and size of LDs in skeletal muscles of type 2 diabetic patients. We hypothesized that volumetric content of LDs will be lowered by training via a reduction in LD size; with greater adaptations in type 2 fibers following HIIT.
Methods

Ethical approval

The study was approved by the Ethics Committee of the Capital Region of Denmark (H-2-2011-070) and was conducted in accordance with the principles of the Declaration of Helsinki. All participants gave their signed informed consent at the start of the study.

Participants

This study is part of a larger randomized controlled trial (NCT02001766) reported in a companion paper (61). All participants completed a standardized medical examination, including blood chemistry analysis, resting 12-lead electrocardiogram and oral glucose tolerance test (OGTT) before inclusion. Exclusion criteria included use of exogenous insulin, smoking, unstable weight (> 5 kg change) in the past 6 months, evidence of renal, liver or cardiovascular disease, and contraindication for physical training. Sixteen out of 29 participants from the larger study, whose muscle biopsies were available for electron microscopy analysis, were included in this study. They were randomized into the Endurance Training (END, n = 8, 4 males, 4 females) and the High-Intensity Interval Training (HIIT, n = 8, 5 males, 3 females) groups.

Experimental protocol

Two experimental days (A and B), separated by 48 h, were conducted before (Pre) and repeated after (Post) the exercise training interventions. Experimental Day A was conducted at least 48 h after the last training session. Participants were instructed to refrain from alcohol and caffeine consumption for 24 h prior to any experimental day. They were also instructed to avoid physically-demanding activity (e.g., bicycle commuting, gardening) for 24 h before Day A and to refrain from taking their anti-diabetic medications on all experimental days.

On Day A, body mass was measured on a digital scale and body fat was assessed by Dual-energy X-ray Absorptiometry. After 15 minutes of supine resting, blood pressure was recorded and baseline blood samples were drawn for determination of plasma glucose, HbA1c, lipids, C-peptide and serum insulin levels. Subsequently, an OGTT (75 g glucose in 300 ml water) was conducted with blood sampling at T = 0, 10, 20, 30, 60, 90, 120 min. Insulin resistance was represented with one of the insulin sensitivity indices – Matsuda index which reflects whole body insulin sensitivity and is calculated from fasting and mean plasma insulin and glucose values during the OGTT (39).
\[ \text{Matsuda index} = \frac{10000}{\sqrt{(\text{fasting glucose} \times \text{fasting insulin}) \times (\text{mean glucose} \times \text{mean insulin})}} \]

Assessments of cardiorespiratory fitness (peak oxygen consumption, \( \dot{V}O_{2\text{peak}} \)), peak workload (peak watt, \( W_{\text{peak}} \)) and heart rate with an incremental cycling protocol were performed last.

On Day B, a resting muscle biopsy was taken before the conduct of additional assessment of glycemic control (mixed meal tolerance test) which was reported in the main randomized controlled trial but not in this study. At the end of Day B, a 4-day continuous glucose monitoring (CGM) protocol and dietary recording were started.

Training interventions

Over 11 weeks, all participants trained 3 times per week on cycle ergometers, with the END group exercised for 40 min at a constant intensity of 50% \( W_{\text{peak}} \) while the HIIT group exercised for 20 min, alternating between 95% \( W_{\text{peak}} \) and 20% \( W_{\text{peak}} \) at 1 min each. Heart rate was monitored at every training session (warm-up and exercise period) and exercise energy expenditure was calculated based on prescribed work load and American College of Sports Medicine’s equations (20). Peak workload was re-assessed after 4 and 8 weeks of training to maintain relative workload during the intervention period.

Muscle biopsies

Skeletal muscle biopsies were taken from m. vastus lateralis using a modified Bergström needle with suction under local anesthesia (2% lidocaine). Biopsied specimens were dissected free of adipose tissue and blood before partitioning into multiple portions; one portion was fixed with a 6.5% (v/v) glutaraldehyde solution with 0.1 mol/l sodium cacodylate-HCl buffer (pH 7.4) for transmission electron microscopy, another portion was freeze-dried for Western blot.

Western blot procedure

Freeze-dried muscle tissue samples were homogenized in lysis buffer (10% glycerol, 20 mM sodium-pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% Nonidet P-40, 20 mM \( \beta \)-glycerophosphate, 10 mM NaF, 2 mM PMSF, 1 mM each EDTA and EGTA, 10 μg/ml each...
aprotinin and leupeptin and 3 mM benzamidine) two times for 30 s (Qiagen Tissuelyser II, Qiagen GmbH, Germany). After rotation end over end for 1 h, the samples were centrifuged for 30 min at 17,500 g at 4°C, and the lysate was collected as the supernatant. Protein concentrations were determined in the lysates (assayed in triplicate) using BSA standards (Pierce Reagents, Thermo Fisher Scientific, MA, USA). The lysates were diluted to appropriate protein concentrations (2 µg µl⁻¹) in a ×6 sample buffer (0.5 M Tris-base, DTT, SDS, glycerol, and bromphenol blue), and equal amount of total protein were loaded for each sample in different wells on pre-casted gels (Bio-Rad Laboratories, CA, USA). After gel electrophoresis, the proteins were transferred to a polyvinylidene difluoride membrane, which was incubated with primary antibody (total OXPHOS, 1:1500, ab110411, Abcam, UK) overnight, and the secondary horseradish peroxidase (HRP)-conjugated antibody (P-0447, Dako, Denmark) 1:5000 in 2/5% non-fat milk or 3% BSA. The membrane staining was visualized by incubation with a chemiluminescent HRP substrate (Millipore) and the image was digitalized on a ChemiDoc MP system (Bio Rad Laboratories, CA, USA). The OXPHOS proteins were normalized to their protein content determined by quantification of each sample on stain free gels (Criterion TGX Stain-Free pre cast gels (BioRad Laboratories, CA, USA).

Transmission electron microscopy

Chemically-fixed muscle biopsy specimens were divided into 2 - 3 pieces (~ 0.5 mm³ each) before post-fixing with 1% (w/v) osmium tetroxide (OsO₄), followed by embedding in Epon 812.

Ultra-thin (60 nm) sections (using a Leica Ultracut UCT ultramicrotome) were made at three depths separated by 150 µm, in order to sample more muscle fibers for imaging. We contrasted the sections with uranyl acetate and lead citrate before examination and capture of electron micrographs in a pre-calibrated JEOL-1400plus transmission electron microscope (JEOL Ltd., Japan) with a CCD camera (Quemesa, EMSIS GmbH, Germany). Thirty electron micrographs were obtained at × 6 000 magnification in a systematic random order, including 18 from the subsarcolemmal region, 6 from the superficial region and 6 from the central region of the myofibrillar space in each fiber. Each electron micrograph covers an area of 9.6 µm by 6.4 µm, which is the minimum interval between micrographs. This protocol was based on the minimum area and length of interest required to maximize the precision of our stereological estimates (41).

Estimation of volume fraction, size and number of lipid droplets and mitochondria
Prior to estimating the lipid droplet and subsarcolemmal mitochondria volume fractions, muscle fibers (8 – 10 per biopsy) were categorized as type 1 or 2, based on a distinct differentiation in terms of intermyofibrillar mitochondrial volume fraction and Z-line width, which was shown to relate to myofibrillar ATPase properties (50). The fibers with the highest intermyofibrillar mitochondrial volume fraction and thickest Z-line width were categorized as type 1 fibers and vice versa for type 2 fibers \( (n = 2 - 3 \) fibers of each type per biopsy). 

Point-counting for the estimation of volume fractions of mitochondria and LDs identified in electron micrographs were performed with reference to stereological principles (59). Identification of mitochondria in micrographs was based on previous reported work on mammalian skeletal muscles (25). Our criteria for identifying LDs included having a circular white-greyish appearance with a fuzzy border (absence of distinct membrane) and a minimum diameter of 200 nm. We included LDs that may have membrane-like structures or irregular partitioning of areas with low (cholesterol esters) or high (triacylglycerol) electron density within the droplets (19).

A point grid (180 nm grid size) was overlaid onto each micrograph, generating a total of 1768 points per micrograph for point-counting. Two subcellular localizations of LD and mitochondria volume fractions were defined: (i) intermyofibrillar volume fraction and (ii) subsarcolemmal volume fraction. The volume fractions of intermyofibrillar LDs and mitochondria per myofibrillar volume were estimated by point-counting micrographs from the superficial and central regions of the myofibrillar space. Muscle fibers are assumed to have a cylindrical shape. As the superficial region of a cylinder occupies 3 times more volume than the central region, volume estimates from the superficial myofibrillar space were weighted 3 times more than those from the central myofibrillar space.

The volume fraction of subsarcolemmal LDs and mitochondria per fiber surface area was estimated by point-counting micrographs from the subsarcolemmal region. We measured the fiber length at the base of the subsarcolemmal region seen in the 18 micrographs. Fiber surface area was calculated as the length of the subsarcolemmal region (directly measured) multiplied by the thickness of the ultrathin section (60 nm).

The volume fractions of total LDs and total mitochondria were computed by adding intermyofibrillar and subsarcolemmal volume fractions per myofibrillar space. The subsarcolemmal volume fraction per myofibrillar space was converted from the subsarcolemmal volume fraction per...
fiber surface area by dividing the subsarcolemmal volume fraction per fiber surface area by a factor
of 20 which was derived geometrically based on the assumption that muscle fibers are cylindrical in
shape with a diameter of 80 μm. The relative distribution of subcellular lipid droplet volume
fractions was calculated after the volume fraction of total lipid droplets was obtained.

Lipid droplet size was estimated by measuring and averaging the largest and smallest caliper
diameters of each LD. Each LD size value was also labeled as either in contact or not in contact
with neighboring mitochondria according to the following criteria. Lipid droplet-mitochondria
contact, represented by percentage of lipid droplets in contact with surrounding mitochondria, was
estimated by counting the number of lipid droplets located next (< 15 nm) to surrounding
mitochondria, out of the total number of whole lipid droplets observable in each electron
micrograph.

Number of LDs was derived from calculation: dividing the volume fraction of subcellular LDs by
the mean individual droplet volume of the respective subcellular localization. Assuming that an
individual LD is a sphere, the mean individual droplet volume was calculated using the formula:
\[ V = \frac{4}{3}\pi r^3 \], where \( r \) was the radius based on the average of the largest and smallest caliper
diameters of each LD.

Assuming an even (50:50) fiber type composition of each biopsy, the fiber type-weighted volume
fractions, size and numbers of LDs were computed by averaging values from type 1 and type 2
fibers for each biopsy. This was done prior to the analysis of association between the Matsuda index
and the fiber type-weighted LD variables.

The precision of stereological estimates, represented by the estimated coefficient of error (\( estCE \)),
for the subcellular lipid droplet and mitochondrial volume fractions was calculated as proposed by
Howard & Reed (26).

\[
CE \left( \frac{\Sigma y}{\Sigma x} \right) = \sqrt{\frac{n}{n-1} \left( \frac{\Sigma x^2}{\Sigma x \cdot \Sigma x} + \frac{\Sigma y^2}{\Sigma y \cdot \Sigma y} - \frac{2 \cdot \Sigma(x \cdot y)}{\Sigma x \cdot \Sigma y} \right)}
\]

For each estimate, \( n \) is the number of micrographs, \( x \) is the total points per micrograph (1768
points), \( y \) is the points hitting object of interest.
A total of 5448 electron micrographs from 186 muscle fibers (91 Pre, 95 Post) were analyzed in this study. The median (IQR) ε\text{est}CE for the volume fractions of subsarcolemmal lipid droplets was 0.25 (0.23 - 0.27) at Pre, 0.28 (0.26 - 0.31) at Post, and for the intermyofibrillar lipid droplets it was 0.24 (0.17 - 0.31) at Pre, 0.25 (0.18 - 0.32) at Post. The median (IQR) ε\text{est}CE for the volume fractions of subsarcolemmal mitochondria was 0.17 (0.16 - 0.19) at Pre, 0.15 (0.13 - 0.17) at Post, and for the intermyofibrillar mitochondria it was 0.08 (0.07 - 0.10) at Pre, 0.08 (0.08 - 0.09) at Post.

Point-counting of LDs and mitochondria was conducted by two blinded investigators, where the electron micrographs from different time points, fiber types and training groups were distributed equally between the investigators. The intraclass correlation coefficient was > 0.92 for inter-investigator and > 0.82 for intra-investigator analyses.

Point-counting, caliper diameter measurement and image analysis was conducted with a commercial transmission electron microscopy imaging software, iTEM (EMSIS GmbH, Germany).

Statistical analysis

Quantitative electron microscopy analyses were conducted with each observation based on estimates from 2 - 3 fibers. All interactions or main effects were tested using a linear mixed-effects model with participants as random effect and with time, training group, fiber type as fixed effects. Variables with skewed distributions were transformed before analysis. The assumption of normal distribution of data was tested by the Shapiro-Wilk test, and the assumption of heteroscedasticity was tested by the Breusch-Pagan / Cook-Weisberg test. Interactions or main effects were tested by Wald Chi-squared test. Associations between variables were evaluated using Pearson's correlation coefficient. Significance level was set at α = 0.05. Power analysis showed that 8 subjects in each group will result in 80% power to detect a 22% difference in subsarcolemmal droplet diameter between pre and post the different training interventions. This calculation was based on our previous study showing a mean (SD) 555 (122) nm diameter of subsarcolemmal lipid droplets (40).

Statistical analyses were performed using Stata 15 (StataCorp LP, Texas, USA) and Prism 6 (GraphPad Software Inc., California, USA).
Results

Training variables and energy intake

Participants in the HIIT group trained at a higher intensity (82 vs 77 % peak heart rate, \( P = 0.03 \)) in shorter sessions (25.1 vs 45.3 min, \( P < 0.0001 \)) (Table 1) as compared to the END group. All participants maintained their energy intakes from pre- to post intervention (Table 1).

Subject characteristics

Training lowered body mass (\( P = 0.01 \)) by reducing fat mass (\( P = 0.01 \)) and improved measures of non-fasting glycemic control (\( P \leq 0.02 \)), with no difference between training modalities (\( P > 0.05 \)) (Table 2). HIIT tended to increase relative \( \dot{V}O_{2\text{peak}} \) more than END (\( P = 0.08 \)) with a 21 % increase following HIIT (\( P < 0.001 \)) and a 9 % increase following END (\( P = 0.04 \)) (Table 2).

Sex differences in volumetric content and morphology of LDs and mitochondria at baseline (Pre)

There were in total 9 male and 7 female type 2 diabetic patients in the 2 training groups. The female participants were found to have higher mean (SD) percent body fat than the male participants (41.3 (2.9) % females vs 29.0 (4.5) % males, \( P < 0.0001 \)). There were no sex differences in the relative \( \dot{V}O_{2\text{peak}} \) or the various measures of glycemic control at baseline.

There was no difference between sexes in the volume fractions of subsarcolemmal LDs and intermyofibrillar LDs and mitochondria. However, there was a tendency for a sex x fiber type interaction (\( P = 0.08 \)) in the volume fraction of subsarcolemmal mitochondria whereby geometric means were 1.5 times higher in males than females in type 1 fibers (males vs. females: 197.9 vs. 130.5 \( \mu m^3 \mu m^{-2} \) fiber area \( \cdot 10^3 \), \( P = 0.04 \)) but not in type 2 fibers (males vs. females: 75.0 vs. 66.9 \( \mu m^3 \mu m^{-2} \) fiber area \( \cdot 10^3 \), \( P = 0.56 \)). Furthermore, there was an interaction between sex and fiber type in the relative distribution of subcellular mitochondria (\( P = 0.006 \)). The mean (SD) percentage contribution of subsarcolemmal mitochondrial to total mitochondrial volume fraction was higher in type 1 than in type 2 fibers (15 (4) type 1 vs. 12 (4) type 2, \( P = 0.001 \)) in males, but this was not observed in females (11 (4) type 1 vs. 11 (3) type 2, \( P = 0.66 \)). In addition, it was also higher in males than in females (15 (4) male vs. 11 (4) female, \( P = 0.03 \)) in type 1 but not type 2 fibers (12 (4) male vs. 11 (3) female, \( P = 0.88 \)).

Fiber type differences in volumetric content and morphology of LDs and mitochondria
Regarding volume fractions of LDs (Table 3), no fiber type difference was observed in the subsarcolemmal region (P = 0.52). However, in the intermyofibrillar region the volume fraction of LDs in type 2 fibers was 55% lower than in type 1 fibers (P < 0.0001). Consequently, the contribution of subsarcolemmal LD to total LD volume fraction in type 2 fibers was 2.4 times higher than in type 1 fibers (P < 0.0001, Table 4).

Compared to type 1 fibers, subsarcolemmal LDs in type 2 fibers were 36% lower in numbers (P = 0.0001, Fig. 3a, b) and ~1.4 times larger in diameter before training (P < 0.05) and after END (P < 0.001) (Fig. 2a, b), while intermyofibrillar LDs in type 2 fibers were 60% lower in number (P < 0.0001, Fig. 3c, d) with no difference in droplet diameter (P = 0.13, Fig. 2c, d).

Subsarcolemmal and intermyofibrillar mitochondrial volume fractions were 61% and 50% lower, respectively, in type 2 fibers compared to type 1 fibers (P < 0.0001, Table 3), as fibers were pre-defined based on mitochondrial content. The contribution of subsarcolemmal mitochondria to total mitochondrial volume fraction was lower in type 2 fibers compared to type 1 fibers (P = 0.01, Table 4).

Effect of training on LDs: volume fraction, droplet diameter and number

Subsarcolemmal LDs

There was neither a 3-way (training x fiber type x group) nor a 2-way ((training x fiber type) or (training x group)) interaction detected in the volume fractions of subsarcolemmal LDs (P ≥ 0.11, Table 3). However, there was a main training effect of 36% reduction in the volume fractions of subsarcolemmal LDs (P = 0.003, Table 3).

A 3-way interaction between training, fiber type and group was found in the diameter of subsarcolemmal LDs (P = 0.02, Fig. 2a, b). This was explained by a 26% reduction in type 2 fibers, following HIIT (P < 0.001) but not END, and with no detectable change following END and a tendency for a 20% reduction following HIIT in type 1 fibers (P = 0.07). The number of subsarcolemmal LDs was unchanged following training (P = 0.71, Fig. 3a, b).

Intermyofibrillar LDs

There was neither an interaction nor a main training effect observed in the volume fractions (P ≥ 0.33, Table 3), droplet diameters (P ≥ 0.13, Fig. 2c, d), numbers (P ≥ 0.80, Fig. 3c, d) of intermyofibrillar LDs following training.
Relative distribution of subcellular LDs

There was neither a 3-way (training x fiber type x group) nor a 2-way ((training x fiber type) or (training x group)) interaction detected in the contribution of subsarcolemmal LD to total LD volume fraction ($P \geq 0.13$, Table 4). There was a tendency for a main training effect shown by a reduction in the contribution of subsarcolemmal LD to total LD volume fraction ($P = 0.07$, Table 4).

Effect of training on volume fraction of mitochondria

Subsarcolemmal mitochondria

There was neither a 3-way (training x fiber type x group) nor a training x fiber type interaction detected in the volume fractions of subsarcolemmal mitochondria ($P \geq 0.11$, Table 3). However, there was a training x group interaction ($P = 0.03$) with a 77% increase following HIIT ($P = 0.02$), but not END ($P = 0.79$) (Table 3).

Intermyofibrillar mitochondria

There was neither an interaction nor a main training effect observed in the volume fraction of intermyofibrillar mitochondria following training ($P \geq 0.14$, Table 3).

Relative distribution of subcellular mitochondria

There was neither a 3-way (training x fiber type x group) nor a training x fiber type interaction detected in the contribution of subsarcolemmal mitochondrial to total mitochondrial volume fraction ($P \geq 0.11$, Table 4). However, there was a training x group interaction ($P = 0.03$): at baseline, the contribution seen in the HIIT group was lower than in the END group ($P = 0.006$) and it increased following HIIT ($P < 0.001$), but not END ($P = 0.32$) (Table 4).

Effect of training on mitochondrial complexes

There was no interaction and main time effect detected in the protein content of the five mitochondrial complexes following training ($P > 0.05$, Fig. 4).

Associations between LD diameter or number of LDs and volume fraction of mitochondria

In the subsarcolemmal region of type 1 fibers, the volume fraction of mitochondria was inversely associated with LD diameter before ($R^2 = 0.38$, $P = 0.01$) and after ($R^2 = 0.38$, $P = 0.01$) training.
(Fig. 5a), whereas there was no association between LD diameter or number of LDs and volume fraction of mitochondria at all other localizations and time-points (Fig. 5a, b, c, d).

**Lipid droplet-mitochondria contact**

There was no interaction, main training or group effect observed in the fiber type-specific percentages of LDs in contact with neighboring mitochondria prior to and following training ($P > 0.05$, Table 5). There was a main fiber type effect whereby the percentage in type 1 fibers was ~45% higher than type 2 fibers ($P < 0.0001$).

Across both subcellular localizations, the diameter of individual LDs either in contact or not in contact with mitochondria was measured at baseline with the following median (IQR) values:

- Contacted LDs in type 1 fibers (610 (503-664) nm), in type 2 fibers (714 (593-806) nm);
- Non-contacted LDs in type 1 fibers (549 (494-596) nm), in type 2 fibers (598 (521-707) nm).

There was neither a 3-way (fiber type x localization x contact) nor a localization x contact interaction detected ($P > 0.05$). However, there was a tendency for a fiber type x contact interaction ($P = 0.08$) that in type 2 fibers the diameter of contacted LDs was 19% larger than non-contacted LDs ($P < 0.001$), whereas the diameter of contacted LDs tended to be 11% larger than non-contacted LDs ($P = 0.06$) in type 1 fibers. Overall, LDs in contact with mitochondria were 13% larger in diameter than non-contacted LDs ($P < 0.0001$).

**Associations between insulin sensitivity index and LDs**

No association was found between the Matsuda index, and the volume fraction, the diameter or the number of LDs in the subsarcolemmal and intermyofibrillar regions separately, at baseline or following training.
Discussion

Our key findings were that in type 2 diabetic patients HIIT but not END, increased the volumetric content of mitochondria and reduced the size of individual LDs located in the subsarcolemmal space. Neither HIIT nor END affected intermyofibrillar LDs or mitochondria. This lowering effect of HIIT on subsarcolemmal LD size was evident in type 2 (fast twitch) muscle fibers. Collectively, these findings reveal that HIIT, having half of END training volume and time commitment, improved oxidative capacity and reduced subcellular volumetric content of LDs, particularly in type 2 muscle fibers of type 2 diabetic patients.

Several studies have shown that both END (7, 40, 49) and HIIT (7, 24, 31, 34) were able to augment the oxidative capacity of skeletal muscles. Likewise, a number of studies which employed transmission electron microscopy to examine the effects of mainly END on subcellular LD and mitochondrial distribution in skeletal muscles of sedentary overweight or type 2 diabetic patients have generally reported that in the subsarcolemmal region, training could lower volumetric content of LDs (42 – 80%) with no training-induced change could be detected in the intermyofibrillar region, while volumetric content of mitochondria was increased (11 – 124%) (16, 33, 42, 47). Our observations on volume fractions of LDs following training are in line with these studies, suggesting a greater plasticity in the subsarcolemmal region following an exercise training stimulus, while LD volumetric adaptation in the intermyofibrillar region may require a training period longer than the one used in this study (18, 49). Interestingly, although in this study training did not induce an increase in protein markers of mitochondrial content (OXPHOS) in mixed muscle specimens, which does not support previous studies in overweight/obese populations (31, 34, 40), we did observe a mitochondrial subcellular localization-specific adaptation, with an increased subsarcolemmal mitochondrial volumetric content following HIIT, underscoring the relevance of quantitative electron microscopy in detecting subcellular localization-specific changes following a stimulus. Lipid species in membrane rather than cytosolic fractions has been demonstrated to associate with insulin resistance (4, 11). In addition, insulin resistance is also associated with subsarcolemmal LD estimates (9, 41, 42). Taken together, existing evidence clearly indicates that in untrained individuals possessing metabolic dysfunctions, although the subsarcolemmal space is not predominant in a whole fiber, it is both more closely linked to muscle insulin resistance and more responsive to physical training, compared to the intermyofibrillar space.
This study not only extends from previous studies (33, 41, 47), but to our knowledge, is also the first to examine fiber type differences in LD morphology in the context of type 2 diabetes and report a subsarcolemmal-specific LD size reduction in type 2 fibers following HIIT. Although very little is known about the physiological significance of LD size, it is has been suggested that the higher surface-to-volume ratio of a smaller LD may facilitate lipolysis by providing the lipases an easier access to the lipid core (54, 63). Hence, the smaller LD size observed following HIIT in this study could imply a training-induced adaptation that aids LD utilization in muscle. Even though type 2 fibers are shown to have a lower capacity for glucose transport and handling compared to type 1 fibers (2), they make up a substantial portion of whole muscle in individuals with type 2 diabetes (2, 43). Therefore, it is highly relevant to assess the metabolic adaptations in muscle with a fiber type-specific approach, and to adopt a training modality, in this case HIIT, which is able to engage a greater portion of whole muscle through the recruitment of type 2 fibers.

Here we did not observe a reduction in the volumetric content of subsarcolemmal LDs following END, which is in contrast to one of our earlier reports (42). This discrepancy may be explained by a lower volumetric content prior to training in the present END group, compared to the type 2 diabetic patients in the previous study (median values: 30 – 34 vs. ~50 µm$^3$.µm$^{-3}$.10$^3$). The training-induced decrease in volumetric content of LDs may be partially explained by the reduction in droplet size with the number of LDs remaining unchanged in the subsarcolemmal region of both fiber types following training. This is in contrast to the drop in LD numbers following training observed previously (16, 33, 47), but it may be due to the larger declines in LD volumetric content shown in those studies.

Previous studies of individuals with type 2 diabetes have shown an increase in total mitochondrial content by END (16, 42, 47). Interestingly, while END together with HIIT induced an increase in total volumetric content of mitochondria in the present study, only HIIT was demonstrated to induce an increase in subsarcolemmal mitochondrial content. This could be partially explained by the total exercise duration per week for END did not exceed the recommended guidelines of 150 min per week which was conducted in one of our studies (42). It could also partly because the total exercise duration per week did not increase progressively over the intervention period, unlike previous studies (16, 47). Hence, the END protocol used in this study might not have been sufficient in training volume to induce volumetric mitochondrial adaptations in the skeletal muscles of our participants.
A notable observation in this study was the inverse association between LD size and the volumetric content of mitochondria in the subsarcolemmal region of type 1 fibers, both before and following training (Fig. 5a). Subsarcolemmal mitochondria have been suggested to support energy demand from the nuclei and biological processes at the sarcolemma (23). With increasing subsarcolemmal volumetric mitochondrial content, particularly in type 1 fibers, the higher metabolic needs would likely require more substrate supply from surrounding LDs. If LDs are considered as hubs for fatty acid trafficking (27, 45), higher flux of fatty acids through the LDs may render a lesser likelihood for neutral lipids to accumulate and expand the LDs. However, a possible role of mitochondrial volume in mediating the morphology and distribution of neighboring LDs awaits confirmation by future studies.

Lipid droplets are shown to be located in close proximity to mitochondria in oxidative tissues such as skeletal muscle (25, 48). Such closeness has been suggested to facilitate the transfer and oxidation of fatty acids in the LD-neighboring mitochondria (5, 45), possibly more so during periods of heightened energy demand. The influence of acute exercise on the LD-mitochondria contact is unclear as mixed findings have been reported (15, 28). Although exercise training has been shown to increase the LD-mitochondria contact in previous studies (16, 55), we did not find a change in the percentage of LD-mitochondria contact following training. However, the contact was found to be higher in type 1 compared to type 2 fibers in the present study. This fiber type difference was not observed in one of our previous study on highly-trained cross-country skiers (28).

With the intimacy between LDs and neighboring mitochondria presumably promoting fatty acid oxidation rather than storage (3), the regulation of fatty acid flux between the two organelles is proposed to be mediated by the LD-associated protein perilipin 5, which may be important in the LD-mitochondrial association (58), although this remains controversial (38). Interestingly, we demonstrated for the first time in human skeletal muscles, the size of LDs in contact with mitochondria was larger than non-contacted LDs at baseline. More studies are needed to have a better understanding of this contact between LDs and mitochondria in different metabolic conditions.

Although it is well-recognized that compared to male individuals, female individuals have a greater reliance on whole-body fat oxidation during moderate-intensity endurance exercise (14), whether women have a greater intramyocellular lipid content than men remains to be confirmed with mixed findings from studies thus far (53, 55, 64). Bearing in mind that the female participants in most
studies were young and healthy, here based on quantitative TEM, we did not find a sex difference in the volumetric content of LDs across both subcellular regions in individuals with type 2 diabetes with a mean age above 50 years old. Existing knowledge on sex-specific subcellular mitochondrial content in skeletal muscle is very limited with a recent report showing no difference in subsarcolemmal and intermyofibrillar mitochondrial area fraction between men and women (8). However, in this study the male participants seemed to have a greater subsarcolemmal mitochondrial content in type 1 fibers as compared to the female participants, suggesting that potential sex differences in subcellular distribution of mitochondria are fiber type-specific.

Due to the low number of males and females, we did not examine sex-specific adaptations to the two training modalities. However, given the higher proportion of men in the HIIT group than in the END group combined with the larger baseline volume fraction of subsarcolemmal mitochondria in men than in women, we investigated whether the HIIT-mediated increase in subsarcolemmal mitochondrial volume fraction was confounded by sex. By including sex as a fixed effect in the mixed effect model, we confirmed the two-way interaction that HIIT, but not END mediated an increase in subsarcolemmal mitochondrial volume fraction ($P = 0.03$), suggesting that this HIIT-specific training adaptation was not confounded by a slightly unbalanced sex distribution between the two training modalities.

Previous reports have correlated volumetric content and size of subsarcolemmal LDs with insulin sensitivity which was assessed with the ‘gold standard’ hyperinsulinemic-euglycaemic glucose clamp technique (41, 42). Although the Matsuda index correlates well with the glucose clamp technique, it was created to reflect more of whole-body rather than peripheral insulin sensitivity (39). Moreover, as fibre type composition was not determined in this study, assumption was made to derive fibre type-weighted LD estimates. Therefore, these could be reasons why no association was detected between Matsuda index and the various fibre type-weighted LD estimates.

To conclude, we have demonstrated that exercise training in general improved whole-body glycemic control in type 2 diabetic patients. In addition, low volume HIIT lowers subsarcolemmal LD volumetric content, particularly via a reduction in the size of LDs, with a concomitant increment of mitochondrial volume in type 2 muscle fibers. This highlights the relevance of HIIT as a time-efficient training modality that induces adaptations in subcellular distribution of LDs, possibly improving glucose regulation in the skeletal muscle of type 2 diabetic patients.
Acknowledgements

Karin Trampedach and Susan Bøgebjerg are acknowledged for their excellent technical assistance in transmission electron microscopy.

Funding

The Centre for Physical Activity Research (CFAS) is supported by a grant from TrygFonden. During the study period, the Centre of Inflammation and Metabolism (CIM) was supported by a grant from the Danish National Research Foundation (DNRF55). This study was further supported by the Capitol Region of Denmark, the Novo Nordisk Foundation, grants from the Danish Diabetes Academy funded by the Novo Nordisk Foundation and the Lundbeck Foundation (R208-2015-3220 and R211-2015-3223).

Duality of interest

No competing interest is declared by all authors.

Author contributions

SPM and KMW contributed to the overall conception and design of the study. All authors contributed to the collection, analysis and interpretation of data. H-CEK, JN and NØ drafted the manuscript. All authors read, revised and approved the final version of manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.


17. Dube JJ, Amati F, Stefanovic-Racic M, Toledo FGS, Sauers SE, Goodpaster BH. Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete’s


28. Koh H-CE, Nielsen J, Saltin B, Holmberg H-C, Ørtenblad N. Pronounced limb and fibre type differences in subcellular lipid droplet content and distribution in elite skiers before and


**Figure legends**

**Fig. 1** Overview and representative transmission electron micrographs of lipid droplets and mitochondria in the subcellular regions. (A) Acquisition of electron micrographs was systematically randomized to cover the subsarcolemmal (SS), superficial myofibrillar (SMF) and central myofibrillar regions (CMF) of each longitudinally-oriented fiber. White arrows indicate the sarcolemma. Original magnification 600 x, scale bar = 20 µm. (B) Localizations of subsarcolemmal lipid droplet (star), mitochondria (SMi) and intermyofibrillar lipid droplet (double stars) and mitochondria (Mi). Black arrow indicates sarcolemma. Occasionally lipofuscin can be observed near the sarcolemma. Original magnification 6000 x, scale bar = 2 µm.

**Fig. 2** Pre- and post-training values for individual lipid droplet diameter in the subsarcolemmal region of type 1 (A) and type 2 (B) muscle fibers; and in the intermyofibrillar region of type 1 (C) and type 2 (D) muscle fibers. Values were observations (END: n = 8, HIIT: n = 8) estimated in 2 - 3 fibers of each fiber type. Black circles, Pre; grey circles, Post. Box and whiskers plots represent medians, interquartile range and adjacent values. There is a 3-way training, fiber type and group interaction. †P = 0.04 vs type 1, ††P < 0.001 vs type 1, ‡P = 0.009 vs END type 2 Post, (*P = 0.07 vs HIIT type 1 Pre), **P < 0.001 vs HIIT type 2 Pre.

**Fig. 3** Pre- and post-training values for number of individual lipid droplets in the subsarcolemmal region of type 1 (A) and type 2 (B) muscle fibers; and in the intermyofibrillar region of type 1 (C) and type 2 (D) muscle fibers. Values were observations (END: n = 8, HIIT: n = 8) estimated in 2 - 3 fibers of each fiber type. Black circles, Pre; grey circles, Post. Box and whiskers plots represent medians, interquartile range and adjacent values. Main fiber type effect †P = 0.0001 vs type 1, ††P < 0.0001 vs type 1.

**Fig. 4.** Pre- and post-training values for skeletal muscle mitochondrial oxidative phosphorylation (OXPHOS) protein content and representative Western and stain-free blots. Vertical dividing lines were used in the Western blot image to present lanes from the same gel that was rearranged for presentation purpose. END: n = 8, HIIT: n = 6. Box and whiskers plots represent medians, interquartile range and adjacent values. C I, complex I subunit; C II, complex II subunit; C III, complex III subunit; C IV, complex IV subunit; C V, complex V subunit. No interaction or main effect was observed (P > 0.05).

**Fig. 5** Associations between mean LD size (A) or number of LDs (B) and volume fractions of mitochondria in subsarcolemmal region; mean LD size (C) or number of LDs (D) and volume fractions of mitochondria in intermyofibrillar region of muscle fibers prior (Pre) and after (Post) training. Values were observations (END: n = 8, HIIT: n = 8) estimated in 2 - 3 fibers of each fiber type. Black circles, type 1 Pre (n = 16); grey circles, type 1 Post (n = 16); black triangle, type 2 Pre (n = 16); grey triangle, type 2 Post (n = 16). All lines fitted linearly.
### Table 1. Energy intake and training data

<table>
<thead>
<tr>
<th>Endurance Training (END)</th>
<th>High Intensity Interval Training (HIIT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
</tr>
<tr>
<td>Energy intake (HIIT, n=7)</td>
<td></td>
</tr>
<tr>
<td>Total energy intake (kJ/day)</td>
<td>7755 (6835-8824)</td>
</tr>
<tr>
<td>Carbohydrate (% of total energy intake)</td>
<td>48 ± 11</td>
</tr>
<tr>
<td>Fat (% of total energy intake)</td>
<td>37 (24-42)</td>
</tr>
</tbody>
</table>

Training intensity (% peak heart rate)
- Mean: 77 ± 5
- Recovery period: 85 ± 4
- Interval: 87 ± 4

Training amount
- Training sessions: 31 ± 3
- Training duration per session (min): 45 ± 3 ± 0.7
- Training duration per session (min): 25.1 ± 0.4

Average training energy expenditure per session (kJ) 1443 ± 431

Values are means ± SD unless stated otherwise.

### Table 2. Participant characteristics and changes in VO\textsubscript{peak}, body composition, blood lipids and glycemic control

<table>
<thead>
<tr>
<th>Endurance Training (END)</th>
<th>High Intensity Interval Training (HIIT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
</tr>
<tr>
<td>n (male/female)</td>
<td>8 (4/4)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>Time since diagnosis (years)</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>6</td>
</tr>
<tr>
<td>DPP-4 inhibitor</td>
<td>0</td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>1</td>
</tr>
<tr>
<td>GLP-1 analogues</td>
<td>0</td>
</tr>
<tr>
<td>No medication</td>
<td>1</td>
</tr>
<tr>
<td>VO\textsubscript{peak}</td>
<td>2.3 (1.9-2.8)</td>
</tr>
<tr>
<td>Absolute (l/min)\textsuperscript{a}</td>
<td>44.7 ± 9.1</td>
</tr>
<tr>
<td>Relative (ml min\textsuperscript{a})</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>Peak workload (watt)</td>
<td>168 ± 43</td>
</tr>
<tr>
<td>Body composition</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>28.6 ± 2.5</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>86.4 ± 11.5</td>
</tr>
<tr>
<td>Fat-free mass (kg)\textsuperscript{b}</td>
<td>53.8 (46.0-63.0)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>29.2 ± 6.2</td>
</tr>
<tr>
<td>Visceral fat mass (kg)</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)\textsuperscript{a}</td>
<td>1.4 (0.9-1.7)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.6 ± 1.1</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)\textsuperscript{b}</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Visceral fat mass (kg)</td>
<td>2.7 (2.1-3.6)</td>
</tr>
<tr>
<td>Matsuda Index\textsuperscript{a}</td>
<td>2.9 (2.1-4.0)</td>
</tr>
<tr>
<td>Glycemic control</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>8.4 ± 2.5</td>
</tr>
<tr>
<td>2-hr OGTT glucose (mmol/l)</td>
<td>13.7 ± 3.7</td>
</tr>
<tr>
<td>Maximum OGTT glucose (mmol/l)</td>
<td>17.0 ± 4.1</td>
</tr>
<tr>
<td>HbA\textsubscript{1c} (mmol/mol)\textsuperscript{a}</td>
<td>52 (44-62)</td>
</tr>
<tr>
<td>HbA\textsubscript{1c} (%)\textsuperscript{a}</td>
<td>7.0 (6.2-7.9)</td>
</tr>
</tbody>
</table>

Values are means ± SD unless stated otherwise. * values are geometric means (95% CI), ** values are medians (interquartile range). n = 8.
Table 3. Total, subsarcolemmal and intermyofibrillar volume fractions of lipid droplets and mitochondria before (Pre) and after (Post) training

<table>
<thead>
<tr>
<th>Volume fractions of lipid droplets</th>
<th>Endurance Training (END)</th>
<th>High Intensity Interval Training (HIIT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (µm³/µm² myofibrillar space · 10³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>13.0 (8.2–23.5)</td>
<td>11.7 (7.1–19.9)</td>
<td>15.9 (10.5–18.8)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>6.0 (4.9–12.6)</td>
<td>9.3 (4.1–9.7)</td>
<td>8.7 (4.6–11.5)</td>
</tr>
<tr>
<td>Subsarcolemmal (µm³/µm² fiber area · 10³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>29.9 (16.8–63.3)</td>
<td>24.7 (10.7–45.4)</td>
<td>37.1 (17.9–53.8)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>34.1 (22.7–48.7)</td>
<td>40.1 (22.9–46.0)</td>
<td>44.2 (29.6–71.6)</td>
</tr>
<tr>
<td>Intermyofibrillar (µm³/µm² myofibrillar space · 10³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>10.9 (8.0–21.0)</td>
<td>10.6 (6.3–17.3)</td>
<td>13.4 (8.6–17.0)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>4.3 (2.9–9.0)</td>
<td>6.9 (2.9–7.5)</td>
<td>5.8 (3.1–8.2)</td>
</tr>
<tr>
<td>Volume fractions of mitochondria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (µm³/µm² myofibrillar space · 10³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>67.1 (58.2–80.9)</td>
<td>72.8 (68.7–90.0)</td>
<td>64.5 (56.2–74.5)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>32.3 (27.2–40.5)</td>
<td>32.2 (17.5–44.3)</td>
<td>31.3 (26.3–38.8)</td>
</tr>
<tr>
<td>Subsarcolemmal (µm³/µm² myofibrillar space · 10³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>199.1 (137.5–288.2)</td>
<td>271.2 (200.7–366.3)</td>
<td>136.6 (101.8–183.4)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>89.4 (70.1–114.0)</td>
<td>75.0 (34.9–161.1)</td>
<td>56.9 (38.8–83.5)</td>
</tr>
<tr>
<td>Intermyofibrillar (µm³/µm² myofibrillar space · 10³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>57.5 (51.4–64.6)</td>
<td>60.6 (55.5–72.9)</td>
<td>59.3 (50.3–66.9)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>28.2 (23.4–34.9)</td>
<td>27.5 (15.4–36.5)</td>
<td>28.2 (24.3–35.7)</td>
</tr>
</tbody>
</table>

Values are medians (interquartile range) unless stated otherwise. * values are geometric means (95% CI). n = 8.

Table 4. Relative distributions of lipid droplets and mitochondria at two subcellular locations in skeletal muscles of type 2 diabetic patients before (Pre) and after (Post) Endurance (END) and High-intensity interval training (HIT)

<table>
<thead>
<tr>
<th>Lipid droplets END</th>
<th>Intermyofibrillar</th>
<th>Subsarcolemmal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>90 (89-93)</td>
<td>91 (86-93)</td>
<td>10 (7-11)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>74 (58-84)</td>
<td>79 (73-83)</td>
<td>26 (16-42)</td>
</tr>
<tr>
<td>Subsarcolemmal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIT Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>88 (81-94)</td>
<td>90 (80-92)</td>
<td>12 (6-19)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>68 (65-73)</td>
<td>79 (72-87)</td>
<td>32 (27-35)</td>
</tr>
<tr>
<td>Mitochondria END</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>85 (82-86)</td>
<td>82 (80-85)</td>
<td>15 (14-18)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>85 (84-89)</td>
<td>85 (83-88)</td>
<td>15 (11-16)</td>
</tr>
<tr>
<td>Subsarcolemmal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIT Type 1 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>90 (86-91)</td>
<td>83 (76-89)</td>
<td>10 (8-14)</td>
</tr>
<tr>
<td>Type 2 fibers Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>91 (89-92)</td>
<td>85 (83-90)</td>
<td>9 (8-11)</td>
</tr>
<tr>
<td>Subsarcolemmal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are medians (interquartile range). n = 8.
Table 5. Percentage of lipid droplets in contact with mitochondria in type 1 and 2 human skeletal muscle fibers before (Pre) and after (Post) Endurance (END) and High-intensity interval training (HIIT).

<table>
<thead>
<tr>
<th></th>
<th>Intermyofibrillar</th>
<th>Subsarcolemmal</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td><strong>END</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1 fibers</td>
<td>76 (69 – 83)</td>
<td>74 (60 – 83)</td>
<td>85 (71 – 90)</td>
<td>81 (63 – 91)</td>
</tr>
<tr>
<td>Type 2 fibers</td>
<td>46 (42 – 60)</td>
<td>46 (27 – 70)</td>
<td>54 (43 – 65)</td>
<td>57 (40 – 73)</td>
</tr>
<tr>
<td><strong>HIIT</strong></td>
<td></td>
<td></td>
<td>For both subcellular regions, P&lt;0.0001. Main fiber type effect.</td>
<td></td>
</tr>
<tr>
<td>Type 1 fibers</td>
<td>77 (70 – 88)</td>
<td>73 (57 – 91)</td>
<td>83 (75 – 92)</td>
<td>83 (71 – 91)</td>
</tr>
<tr>
<td>Type 2 fibers</td>
<td>57 (55 – 64)</td>
<td>44 (29 – 60)</td>
<td>61 (55 – 78)</td>
<td>56 (31 – 68)</td>
</tr>
</tbody>
</table>

Values are medians (interquartile range). n = 8, 2122 subsarcolemmal lipid droplets and 3131 intermyofibrillar lipid droplets analyzed.
Type 1

A

Diameter of individual subsarcolemmal lipid droplet (nm)

Pre  Post  Pre  Post

(END)

Type 2

B

††

HIIT

††

‡

C

D

Diameter of individual intermyofibrillar lipid droplet (nm)

Pre  Post  Pre  Post

(END)

HIIT

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Number of subsarcolemmal lipid droplets (droplets $\mu m^{-2}$ fibre area x $10^3$)

Number of intermyofibrillar lipid droplets (droplets $\mu m^{-3}$ myofibrillar space x $10^3$)

††
**Intramitochondrial Protein Localization**

The diagram illustrates the localization of intramitochondrial proteins following different conditions (END and HIIT) before and after intervention.

- END pre: 54kDa C, 48kDa C, 45kDa C
- END post: 54kDa C, 48kDa C, 45kDa C
- HIIT pre: 54kDa C, 48kDa C, 45kDa C
- HIIT post: 54kDa C, 48kDa C, 45kDa C

Protein levels are assessed using stain-free blots for each condition.

**Protein Levels**

- 15kDa
- 20kDa
- 25kDa
- 37kDa
- 50kDa
- 75kDa

**Mitochondrial Enzymes**

- 18kDa C I-NDUFB8
- 22kDa C IV-COX II
- 29kDa C II-SDHB
- 48kDa C III-UQCRC2
- 54kDa C V-ATP5A

**Enzyme Activity**

- C I
- C II
- C III
- C IV
- C V

**Conditions**

- END
- HIIT

**Comparison**

The comparison shows a change in protein levels and enzyme activity pre and post intervention, indicating a possible effect of END and HIIT on intramitochondrial protein localization.
Volume fraction of subsarcolemmal mitochondria (µm$^3$ µm$^{-2}$ fibre area x 10$^3$)

Diameter of individual subsarcolemmal lipid droplet (nm)

Type 2 Post: $R^2=0.01$, $P=0.68$

Type 2 Pre: $R^2=0.04$, $P=0.44$

Type 1 Post: $R^2=0.01$, $P=0.68$

Type 1 Pre: $R^2=0.0001$, $P=0.97$

Volume fraction of intermyofibrillar mitochondria (µm$^3$ µm$^{-3}$ myofibrillar space x 10$^3$)

Diameter of individual intermyofibrillar lipid droplet (nm)

Type 2 Post: $R^2=0.04$, $P=0.44$

Type 2 Pre: $R^2=0.14$, $P=0.15$

Type 1 Post: $R^2=0.008$, $P=0.74$

Type 1 Pre: $R^2=0.0002$, $P=0.96$

Number of subsarcolemmal lipid droplets (droplets µm$^{-2}$ fibre area x 10$^6$)

Type 2 Post: $R^2=0.0001$, $P=0.97$

Type 2 Pre: $R^2=0.06$, $P=0.35$

Type 1 Post: $R^2=0.08$, $P=0.29$

Type 1 Pre: $R^2=0.13$, $P=0.17$

Number of intermyofibrillar lipid droplets (droplets µm$^{-3}$ myofibrillar space x 10$^6$)

Type 2 Post: $R^2=0.08$, $P=0.29$

Type 2 Pre: $R^2=0.02$, $P=0.56$

Type 1 Post: $R^2=0.01$, $P=0.68$

Type 1 Pre: $R^2=0.02$, $P=0.56$