Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is paralleled by increased myocellular fat storage and improved insulin sensitivity.

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**Objective** - Mitochondrial dysfunction and fat accumulation in skeletal muscle (IMCL) has been linked to development of type 2 diabetes. We examined if exercise training could restore mitochondrial function and insulin sensitivity in patients with type 2 diabetes (T2D).

**Methods** - 18 male T2D and 20 healthy male control subjects (C) of comparable body weight, BMI, age and VO2max, performed a 12 week combined progressive training program (3x/week, 45 minutes/session). In vivo mitochondrial function (MRS), insulin sensitivity (clamp), metabolic flexibility (indirect calorimetry) and IMCL content (histochemically) were measured before and after training.

**Results** - Mitochondrial function was lower in T2D compared to C (p=0.03), improved by training in C (+28%, p=0.02) and restored to control values in T2D (+48%, p<0.01). Insulin sensitivity tended to improve in C (delta Rd +8%, p=0.08) and improved significantly in T2D (delta Rd +63%, p<0.01). Suppression of insulin stimulated endogenous glucose production improved in both groups (EGP -64%, p<0.01 in C and -52%, p<0.01 in T2D). After training, metabolic flexibility in T2D was restored (delta RER +63%, p=0.01), but was unchanged in C (delta RER +7%, p=0.22). Starting with comparable pre-training IMCL levels, training tended to increase IMCL content in T2D (+27%, p=0.10), especially in type 2 muscle fibers.

**Conclusion** - Exercise training restored in vivo mitochondrial function in T2D. Insulin mediated glucose disposal and metabolic flexibility improved in T2D in the face of near significantly increased IMCL content. This indicates that increased capacity to store IMCL and restoration of improved mitochondrial function contribute to improved muscle insulin sensitivity.
Skeletal muscle insulin resistance is one of the earliest hallmarks in the development of type 2 diabetes mellitus. The combination of increased intramyocellular lipid (IMCL) and a low oxidative capacity are key features in the development of muscular insulin resistance (1-3). Thus, mitochondrial dysfunction has been suggested to be involved in accretion of IMCL.

In type 2 diabetes, smaller and damaged mitochondria have been reported (4). In line with this, gene expression of a key transcriptional co-factor in mitochondrial biogenesis (PGC1α), and its target genes encoding key enzymes in oxidative mitochondrial metabolism, was lower in (pre-) diabetic subjects (5; 6). We confirmed lower expression of PGC1α in type 2 diabetic patients and a restoration towards control values upon treatment with rosiglitazone (7), indicating that PGC1α mediated defects in mitochondria are reversible.

Importantly, these defects can translate into lower in vivo ATP synthesis rate in first-degree relatives of type 2 diabetic patients, as determined using 31P-Magnetic Resonance Spectroscopy (8). Using an alternative 31P-MRS method, we recently reported that type 2 diabetic patients are also characterized by a reduced in vivo mitochondrial function, as reflected by a prolonged post-exercise phosphocreatine resynthesis rate (9). More recently, we extended this observation with ex vivo data indicating intrinsic mitochondrial defects in patients with type 2 diabetes (10). Under all these conditions, compromised mitochondrial function was observed in overweight to obese, BMI matched populations with a comparable IMCL content. Together, these data support the hypothesis that a low oxidative capacity may contribute to the development of insulin resistance in the presence of a high IMCL content (11).

Current ADA/AHA-based guidelines in prevention and treatment of type 2 diabetes target diet-induced weight loss of 5-10% body weight and physical activity of at least 150 minutes of moderate activity per week (12). Interestingly, although both are insulin sensitizing interventions, diet-induced weight loss and physical exercise training differentially affect IMCL content. While a diet-induced reduction in body mass resulted in declined IMCL content (13), exercise training leads to IMCL accretion (11; 14-16). The net effect of combined dietary and exercise interventions may hence be similar IMCL levels pre- and post-intervention. Indeed, in patients with type 2 diabetes it has been shown that a combined exercise-dietary intervention improved insulin sensitivity without changes in IMCL, but with an improvement in mitochondrial function (13). On the other hand, diet-induced weight loss alone reduces IMCL content, without affecting mitochondrial capacity, suggesting that to improve muscular insulin resistance the balance between IMCL content and oxidative capacity is critical (13).

At present, the effect of exercise without dietary restrictions and without targeted weight loss on IMCL content, mitochondrial function and insulin sensitivity is unknown. In addition, it is not known if the response to exercise training in type 2 diabetes -with respect to insulin sensitivity, mitochondrial content and function and IMCL- differs from the response in BMI matched normoglycemic control subjects.

Therefore, we aimed to investigate the effect of a well-controlled 12 week training program in type 2 diabetic patients and carefully matched obese healthy controls on insulin sensitivity, in vivo mitochondrial function and content, and intramyocellular lipid content.

RESEARCH DESIGN AND METHODS
Subjects characteristics: 18 male type 2 diabetic subjects (T2D) and 20 healthy male control subjects (C) matched for body weight, body mass index (BMI) and age were included. Exclusion criteria were cardiac disease, impaired liver or renal function, BMI>35 kg/m², diabetic complications, exogenous insulin therapy and prior participation in training studies. For control subjects, a family history of type 2 diabetes was added to the exclusion criteria. Glucose tolerance was examined by an oral glucose tolerance test (OGTT) (17). Diabetic patients were diagnosed with type 2 diabetes for at least 1 year before the start of the study, had well-controlled diabetes (HbA1c = ± 7.2%) and were using oral anti-diabetic agents (metformin only, or in combination with SU derivatives). Medication use did not change during the study. None of the subjects were on a diet and all had a sedentary lifestyle. The institutional medical ethical committee approved the study. Body composition was measured through hydrostatic weighing (18), and maximal work load and oxygen uptake was assessed during a graded cycling test until exhaustion (19).

Exercise training protocol: Subjects enrolled in a tightly controlled exercise program for 12 weeks. Aerobic exercise was carried out on a cycling ergometer twice a week for 30 minutes at 55% of a previously determined maximal work load (Wmax). Resistance exercise was performed once a week and comprised 1 series of 8 repetitions at 55 % of their previously determined maximal voluntary contraction (MVC) and 2 series of 8 repetitions at 75 % MVC and focussed on large muscle groups (Chest press, leg extension, lat pull down, leg press, triceps curls, biceps curls, abdominal crunches, horizontal row). The MVC test was preceded by a familiarization trial. Warming-up and cooling-down sessions of 5 minutes were performed on a stationary bike at 45% Wmax. Every 4 weeks, MVC was re-assessed; maximal aerobic capacity was re-assessed after 6 weeks and training loads were readjusted accordingly. Supervised training sessions were performed with 4 subjects at a time.

Hyperinsulinemic-euglycemic clamp: A 6-hour hyperinsulinemic-euglycemic insulin clamp (40mU/m²/min) was performed before and after the training period essentially as described previously (7). Dietary habits were stable and physical exercise was avoided 3 days prior to the clamp. Diabetic subjects discontinued anti-diabetic medication 7 days prior to the clamp. Glucose tracer ([6,6-2H2]glucose) was used to determine rates of glucose appearance (Ra) and disposal (Rd). The first three hours (t=0-180 min) were used to determine non-insulin stimulated Ra and Rd. At t=180, a primed constant infusion of insulin started and glucose infusion rates was adjusted to maintain euglycemia. In the final 30 minutes of the non-insulin stimulated period (t=150-180) and under steady clamp conditions (t=330-360), blood was sampled and indirect calorimetry (ventilated hood) was performed. Muscle biopsies from the m. vastus lateralis were obtained under local anaesthesia (2%lidocaine), before and after the clamp.

Tracer calculations: Isotopic enrichment of plasma glucose was determined by electron ionization gas chromatography–mass spectrometry. Steele’s single-pool non–steady-state equations (20) were used to calculate glucose Ra and Rd. Volume of distribution was assumed to be 0.160 l/kg for glucose. Insulin stimulated glucose disposal was computed as the difference between Rd under insulin stimulated conditions and Rd under basal non-insulin-stimulated conditions (delta Rd). Endogenous glucose production (EGP) was calculated as Ra minus exogenous glucose infusion rate. Non-oxidative glucose disposal was calculated as Rd minus carbohydrate oxidation.
Blood sample analysis: Arterialised blood samples were collected from a hand vein. Plasma free fatty acids (FFAs) and glucose were measured spectrophotometrically. Insulin concentration was determined using a radioimmunoassay (Linco Research, St. Charles, MO).

Metabolic flexibility: Fat and carbohydrate oxidation in the basal and insulin stimulated state was calculated according to Frayn (21) with protein oxidation considered negligible. Metabolic flexibility was expressed as the change in respiratory exchange ratio (RER) from the fasted state to the insulin-stimulated condition.

$^{31}P$-MRS-based measurement of mitochondrial function: $^{31}P$-MRS measurements were performed in vastus lateralis muscle on a 1.5 T whole body scanner (Intera, Philips Health Care, Best, the Netherlands) essentially according to (9). Data are expressed as rate constant (ln 2/ Pr recovery time in seconds). The higher the rate constant (s$^{-1}$), the better in vivo mitochondrial function.

Intramyocellular lipid content: Intramyocellular lipid (IMCL) content was assessed histochemically in muscle cross-sections using a modified oil red O staining for fluorescence microscopy (28) and combined with muscle fiber typing.

Western blotting: UCP3 protein content was determined by Western blotting, using a rabbit polyclonal antibody. Five different structural components of the electron transport chain were measured at the protein level as a reflection of mitochondrial density. The components include ND6 subunit of complex I, the 30 kDa Ip subunit of complex II, the 47 kDa core protein 2 of complex III, subunit II of cytochrome C oxidase (COXII) and the alpha subunit of the F1F0 ATP synthase (complex V) and were measured using monoclonal antibodies (MitoSciences, Oregon, USA) (29). Gels were loaded with equal amounts of protein of pre- and post training lysates of 2 control and 2 type 2 diabetic subjects per gel to allow valid comparison between pre- and post training samples. To adjust for inter-gel variation, and hence variation in the mean of the control group and the type 2 diabetic group, the optical density of the band of interest per subject was normalized to the mean optical density of the complete gel. Protein content was expressed as arbitrary units (AU).

Statistics: Data are presented as mean ± SE. In 4 control and 3 T2D no pre- or post data for MRS analyses could be obtained due to claustrophobia or metal parts in their body. Statistical analyses were performed two-sided using SPSS for Windows 15.0 software (SPSS, Chicago,IL. Statistical significance was set at p < 0.05. A two-way ANOVA model for repeated measures was applied using control and type 2 diabetics as between subject variables and pre- and post training data as repeated within subject variables. Using this model we did not find significant interaction effects.

RESULTS

Subjects characteristics: Control subjects (C) and type 2 diabetic patients (T2D) were included and matched for body weight (94.7 ± 2.7 and 93.8 ± 2.9 kg), BMI (29.7 ± 0.8 and 30.0 ± 0.8 kg/m$^2$) and age (59.0 ± 0.8 and 59.4 ± 1.1 years in C and T2D). Training induced a modest but near significant decline in body weight and BMI in C (94.7 ± 2.7 to 93.6 ± 2.7 kg and 29.7 ± 0.8 to 29.4 ± 0.8 kg/m$^2$ respectively, both p=0.06), but did not result in significant changes in T2D (table 1). Fat mass tended to decline in C (30.0 ± 1.8 kg to 29.2 ± 0.2 kg after training; p=0.09) and declined modestly but significantly after training in T2D (29.4 ± 1.9 kg to 28.0 ± 1.8 kg; p=0.04). Fat free mass was similar between both groups and did not change after training (table 1).

Exercise capacity: Before training, maximal oxygen uptake was comparable in both
groups and increased upon training (+6.4 ± 2.6%, p=0.04 in C and +11.3 ± 2.2%, p<0.01 in T2D). Maximal oxygen uptake and \( W_{\text{max}} \) increased similarly in C and T2D (+15.0 ± 2.4% and +16.9 ± 3.0% in C and T2D respectively, p<0.01) and persisted after correction for body mass. Resistance training profoundly improved muscle strength (+22.2 ± 2.0% and +23.9 ± 1.9% in C and T2D respectively, p<0.01, table 1).

**Whole-body insulin-stimulated glucose disposal:** Insulin-stimulated glucose disposal (delta \( R_d \)) was considerably higher in C than in T2D (table 2). Training induced a near significant increase in delta \( R_d \) in C (17.1 ± 2.4 to 18.4 ± 2.1 \( \mu \text{mol/kg/min} \), p=0.08) and a profound significant increase in T2D (from 6.8 ± 1.4 to 11.1 ± 1.4 \( \mu \text{mol/kg/min} \), p<0.01). The significant increase observed in patients with type 2 diabetes originates from a reduction in basal glucose disposal rate as well as from improved disposal rate under insulin stimulated conditions (table 2). Nevertheless, insulin-stimulated glucose disposal remained higher in C after training compared to T2D. Basal endogenous glucose production before training tended (p=0.07) to be lower in C than in T2D. Insulin sensitivity of the liver in C and T2D improved significantly (endogenous glucose production drops from 2.8 ± 0.8 pre-training to 1.0 ± 1.0 in C, p=0.01 and from 2.9 ± 0.5 pre-training to 1.4 ± 0.3 \( \mu \text{mol/kg/min} \) in T2D, p<0.01). Insulin-mediated non-oxidative glucose disposal was significantly higher in C than in T2D and was not significantly affected by exercise training (p=0.14 and 0.13 in C and T2D respectively, table 2). In contrast, delta glucose oxidation in T2D restored to control values (table 2).

**In vivo mitochondrial function by \( ^{31}\text{P-MRS} \):** We confirmed compromised mitochondrial function in T2D compared to BMI matched controls (rate constant: 0.036 ± 0.002s\(^{-1} \) and 0.030 ± 0.001s\(^{-1} \) in C and T2D respectively, p=0.03). Mitochondrial function improved after training in C and T2D (+28% and +48% respectively), resulting in similar in vivo mitochondrial function after training (p=0.84) (figure 1). There was no difference in end-exercise pH values between the C and T2D and no difference between pre- and post training values (7.05 ± 0.02 and 7.02 ± 0.02 for C pre- and post-training and 7.01 ± 0.02 and 6.99 ± 0.03 for T2D pre- and post training respectively).

**Metabolic flexibility:** Prior to training, metabolic flexibility was significantly higher in control subjects compared to T2D (figure 3), due to a more profound increase in insulin stimulated glucose oxidation (5.9 ± 0.7 and 3.6 ± 0.8 \( \mu \text{mol/kg/min} \) in C and T2D respectively, p=0.04), with a concomitant tendency (p=0.10) to more profound insulin-mediated suppression of fat oxidation (-0.46 ± 0.05 and -0.32 ± 0.06 \( \mu \text{mol/kg/min} \) in C and T2D respectively) (table 2). Exercise training did not affect metabolic flexibility in C but fully restored flexibility in T2D (C versus T2D; p=0.84, figure 3), due to improved insulin stimulated increases in glucose oxidation (from 3.61 ± 0.78 to 5.94 ± 0.72 \( \mu \text{mol/kg/min} \); p=0.02) and suppression of fat oxidation (from -0.32 ± 0.06 to -0.49 ± 0.06 \( \mu \text{mol/kg/min} \); p = 0.01) (table 2). Remarkably, insulin-stimulated substrate oxidation rates were comparable between T2D and C after training, indicating that the lower insulin-stimulated glucose uptake after training in T2D was completely accounted for by a lower insulin-stimulated non-oxidative glucose uptake.

**Intramyocellular lipid content:** IMCL content pre-training was comparable in both groups and did not change after training in C (figure 2). In T2D however, training tended to increased IMCL content (from 1.5 ± 0.2 to 1.9 ± 0.3AU; p=0.10) which was predominantly accounted for by a near significant increase in IMCL in type 2 muscle fibers (from 1.1±0.2 to 1.5±0.3AU; p=0.07, figure 2).
Blood sample analysis: Fasting glucose levels and HbA1c were significantly lower in C than in T2D prior to the training program. Twelve weeks of exercise training induced a slight but significant decrease in HbA1c and in fasting glucose levels in C. There was no change in HbA1c or fasting glucose levels in T2D (table 1). Fasting plasma insulin levels were comparable pre-training and decreased significantly in both groups after training (table 2). Fasting plasma free fatty acid (FFA) levels were similar across groups and did not change after training. Under insulin stimulation, however, FFA levels tended to be lower in C compared to T2D and decreased significantly after training in both groups (table 2), suggesting improved insulin mediated suppression of adipose tissue lipolysis post-training in both groups.

Markers of mitochondrial density: Mitochondrial density was evaluated by measuring protein content of 5 structural subunits of the distinct complexes of the electron transport chain. Neither the individual complexes (table 3), nor averaging the protein content of these complexes revealed difference in mitochondrial density between C and T2D pre-training (0.66 ± 0.11 and 0.59 ± 0.08 AU in C and T2D, NS). Training resulted in increased mitochondrial density in both groups after training (table 3). The increase in mitochondrial density tended to be more pronounced in T2D compared to C (p=0.07).

UCP3 protein content: We confirm our previous reports showing a ~50% lower UCP3 content in T2D than in C (30; 31) (table 3). Training increased UCP3 protein content two-fold in C and almost 4-fold in T2D. This resulted in similar UCP3 content in C and T2D after training (table 3).

DISCUSSION
Mitochondrial dysfunction has been reported in type 2 diabetic patients (4; 32; 33) and in young, lean, insulin-resistant offspring of parents with type 2 diabetes (2), although not all studies support this (34; 35). The present study confirms our previous observation of compromised mitochondrial function measured in vivo in patients with type 2 diabetes (9; 10). Importantly, exercise training in patients with type 2 diabetes completely restored mitochondrial function towards values observed in control subjects after training. In patients with type 2 diabetes, restoration of mitochondrial function was paralleled by improved (but not restored) insulin stimulated glucose disposal and by complete restoration of metabolic flexibility and insulin-stimulated substrate oxidation towards control values, both in face of a near significant increase in IMCL content. In control subjects, training also improved mitochondrial function while insulin stimulated glucose disposal increased only marginally and metabolic flexibility and IMCL content remained unaltered.

The ability of patients with type 2 diabetes to increase mitochondrial function indicates that despite aberrations in transcriptional control of mitochondrial biogenesis (5; 6), a lifestyle intervention comprising physical exercise is potent enough to overcome these apparent defects. Increased mitochondrial content and improved function has previously been observed in type 2 diabetes following a combined dietary exercise intervention targeting more than 7% body weight loss (13). Here we show that exercise training, even without substantial loss of body mass, not only improves mitochondrial function but even results in complete restoration towards control values observed in age and BMI matched normoglycemic control subjects. The observation of compromised mitochondrial function in patients with type 2 diabetes compared to control subjects, whilst having comparable mitochondrial density (as indicated by measuring protein content of 5 structural components in the electron transport chain), supports previous findings of
intrinsic defects in mitochondria of patients with type 2 diabetes (10; 36). Interestingly, mitochondrial protein content markedly increased after exercise training, suggesting that at least a major part of the restoration of mitochondrial function after training is due to increased mitochondrial biogenesis. Although it remains to be established if exercise training also improves intrinsic mitochondrial function, it is of interest to note that protein expression of UCP3, a protein with a putative role in ameliorating lipotoxicity and oxidative stress via mild uncoupling (37) was significantly lower in type 2 diabetic patients compared to controls, confirming previous work (30; 31). UCP3 content restored to control values after training in type 2 diabetes, even after adjustment for the increase in structural components of the electron transport chain. This may indicate that exercise training in patients with type 2 diabetes not only improves mitochondrial content but also results in adaptive responses within mitochondria to cope better with the myocellular metabolic stress in the insulin resistant state.

Part of the metabolic stress in type 2 diabetes may originate from myocellular fat storage. IMCL content correlates negatively with insulin sensitivity in untrained subjects (11; 38; 39). On the other hand, endurance trained athletes also have high levels of IMCL (11; 40), while being insulin sensitive. It has hence been suggested that low fat oxidative capacity and a concomitant increase in fatty acid metabolites induces insulin resistance, rather than IMCL levels per se (11; 41). Our present study confirms previous findings of reduced mitochondrial function in T2D with a similar IMCL content between control subjects and type 2 diabetic patients (9; 10). This suggests that high IMCL levels combined with compromised mitochondrial function may contribute to impeded insulin sensitivity. This notion is substantiated by our observation that exercise training improved mitochondrial function and alleviated muscular insulin resistance in patients with type 2 diabetes even though IMCL levels increased post-training.

Training-induced increases in IMCL content may originate from improved partitioning of fatty acids in IMCL due to exercise-induced increases in diacylglycerol-acyl transferase (DGAT1) (42; 43), the rate limiting enzyme in IMCL synthesis. Indeed enhancing IMCL storage capacity by overexpression of DGAT1 improved insulin sensitivity (42). These findings support the idea that the capacity to effectively store fatty acids as IMCL along with appropriate mitochondrial function are major determinants of myocellular insulin sensitivity. We observed increased IMCL content in type 2 diabetic patients after combined endurance and resistance training in glycolytic type 2 muscle fibers, which in human posses lower IMCL levels than the more oxidative type 1 fibers. It could hence be suggested that, due to the resistance exercise, previously inactive type 2 fibers were now recruited and increased their storage capacity for fatty acids as IMCL, thereby contributing to the insulin sensitizing effect of training. This implies that it might be of added value for insulin sensitizing training interventions to include also exercise at an intensity which requires recruitment of type 2 muscle fibers.

Metabolic inflexibility is another characteristic of insulin resistant muscles (44), possibly reflecting a reduced ability of mitochondria to shift fuel selection. Metabolic inflexibility in insulin resistance may reflect reduced insulin stimulated glucose uptake, thereby reducing the availability of glucose for oxidation rather than a mitochondrial defect in substrate selection (45). The present study partly supports this notion. Impaired metabolic flexibility in T2D before training was indeed accompanied by a reduced insulin-stimulated rate of glucose disappearance. Moreover, upon training,
insulin stimulated glucose disposal improved in the T2D in conjunction with improved metabolic flexibility. Although the improvement in insulin-stimulated glucose disposal completely matched the restoration of metabolic flexibility, restoration of mitochondrial function may be needed to facilitate this. In control subjects, training did not alter metabolic flexibility and also only marginally improved insulin-stimulated glucose disposal. It thus seems that after training, insulin-stimulated glucose oxidation was working at its maximal capacity in both C and T2D. Very interestingly, despite a restoration of metabolic flexibility, mitochondrial function and insulin-stimulated glucose oxidation, insulin-stimulated glucose disposal was still lower in T2D compared to C. This was completely accounted for by a lower non-oxidative glucose disposal. Thus, upon exercise training the oxidative component of insulin-stimulated glucose disposal is fully restored, in contrast to non-oxidative glucose disposal. Compromised non-oxidative glucose disposal in type 2 diabetes has been reported previously (46) and treating insulin resistant first-degree relatives of type 2 diabetes with metformin normalizes non-oxidative glucose disposal (47), supporting the notion that restoring non-oxidative glucose disposal may be crucial to normalize insulin sensitivity and possibly plasma glucose in type 2 diabetes. 

In a model of one-legged exercise training, non-oxidative glucose disposal improved along with increased fractional velocity of glycogen synthase (48). The different training regimes applied (one vs. two-legged exercise, 6 times per week vs. 3 times per week, and aerobic exercise solely vs. a combination of aerobic and resistance exercise) in the one-legged exercise study vs. the present study are likely to explain the differences. It should be noted that also in the present study, in patients with type 2 diabetes, non-oxidative glucose disposal improved with ~30%, albeit non-

significantly but was still lower than control values. More recently, restoration of non-oxidative glucose disposal upon exercise training in type 2 diabetes has been reported (49). In that study, however, non-oxidative glucose disposal was measured as the residual of glucose disposal rate minus oxidative glucose disposal and may hence be biased by hepatic glucose production and therefore are hard to compare to the data from the present study. Future studies are needed to examine the underlying mechanisms, and to examine exercise training could be combined with other means augments non-oxidative glucose disposal and thereby further improve insulin sensitivity.

While skeletal muscle insulin resistance is the hallmark of type 2 diabetes, insulin resistance of liver and adipose tissue also contributes to the pathogenesis of type 2 diabetes. In this respect it is of relevance to note that physical exercise training also resulted in beneficial adaptations beyond those reported for muscle. Likewise we observed that under hyperinsulinemic clamp conditions plasma free fatty acid levels were significantly lower post- than pre-training, possibly reflecting improved anti-lipolytic activity of insulin in adipose tissue. In addition, exercise training in type 2 diabetic patients improved the ability of insulin to inhibit hepatic glucose output. At present, the routes or mechanisms responsible for these beneficial training-mediated multiple organ adaptations are unknown and warrant further study.

In conclusion, restoration of mitochondrial dysfunction in type 2 diabetes by physical exercise improves insulin mediated glucose disposal in the presence of increased IMCL storage. Restoration of mitochondrial function and metabolic flexibility in type 2 diabetes by exercise is at least partly accounted for by increased mitochondrial content and possibly intrinsic mitochondrial adaptations. The insulin sensitizing effect of physical exercise training occurs in the absence of major
changes in body mass and is not restricted to improved muscle insulin sensitivity but extends to improved hepatic- and adipose tissue insulin sensitivity.

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Figure legends

Figure 1: In vivo mitochondrial function measured in vastus lateralis muscle expressed as rate constant (s⁻¹), before (black bars) and after training (white bars). A high rate constant reflects high in vivo mitochondrial function. Data are expressed as mean ± SE., #T2D significantly different from control group, *post-training significantly different from pre-training. Pre- and post training leg extension exercise was performed at 0.5 Hz to an acoustic cue on an MR compatible ergometer and a weight corresponding to 60% of the predetermined maximum. Spectra were fitted in the time domain with the AMARES algorithm (22) in the jMRUI software (23). Five peaks were fitted with gaussian curves (Pi, PCr, and 3 ATP peaks). The time-course of the PCr amplitude (PCr(t)) during the last 20 seconds of exercise (steady state) and during the recovery period was fitted as described previously (9), assuming a monoexponential PCr recovery. Post-exercise, PCr resynthesis is driven almost purely oxidatively (24) and the resynthesis rate reflects in vivo mitochondrial function in health (25) and disease (for reviews see (26; 27).

Figure 2: Intramyocellular lipid content in all muscle fibers (panel 2a), in type 1 muscle fibers (panel 2b) and in type 2 muscle fibers (panel 2c). Data are expressed as mean ± SE., #T2D significantly different from control group, *post-training significantly different from pre-training. Muscle fiber typing was performed using a monoclonal primary antibody against the slow isoform of myosin heavy chain 1 (MHC1), which was visualized using a secondary FITC conjugated secondary antibody. Thus, MHC1 positive cells were considered type 1 muscle fibers whereas MHC1 negative cells were considered type 2 muscle fibers. Immunolabelling of the basement membrane protein laminin was performed to identify the cellular border. Thus, we were able to identify the typology of individual muscle cells. Tresholding the oil red O signal allowed us to compute the relative fraction of cell area containing lipid droplets per individual muscle fiber of either type.

Figure 3: Metabolic flexibility, measured as the change in respiratory quotient from the fasted state to the insulin-stimulated state, before (black bars) and after training (white bars). Data are expressed as mean ± SE., #T2D significantly different from control group, *post-training significantly different from pre-training.
Table 1: subject characteristics

| Subject characteristics | Pre-training C | Post-training C | Pre-training T2D | Post-training T2D |
|-------------------------|----------------|-----------------|-----------------|------------------|
| Age (years)             | 59.0 ± 0.8     | 59.4 ± 1.1      |                 |                  |
| Years since diagnosis   | /              | 3.9 ± 0.9       |                 |                  |
| Weight (kg)             | 94.7 ± 2.7     | 93.6 ± 2.7      | 93.8 ± 2.9      | 92.8 ± 3.1       |
| Height (cm)             | 178.5 ± 1.3    | 176.7 ± 1.3     |                 |                  |
| BMI (kg/m²)             | 29.7 ± 0.8     | 29.4 ± 0.8      | 30.0 ± 0.8      | 29.8 ± 0.9       |
| Body fat (%)            | 31.5 ± 1.4     | 30.6 ± 1.6      | 31.1 ± 1.4      | 29.9 ± 1.3 *     |
| FM (kg)                 | 30.0 ± 1.8     | 29.2 ± 2.0      | 29.4 ± 1.9      | 28.0 ± 1.8 *     |
| FFM (kg)                | 64.6 ± 2.0     | 65.4 ± 2.0      | 64.3 ± 1.7      | 64.8 ± 1.8       |
| VO2max (ml/min/kg)      | 28.8 ± 1.0     | 30.2 ± 1.2 *    | 27.5 ± 1.2      | 31.1 ± 1.2 *     |
| Wmax (Watt)             | 207 ± 10       | 236 ± 9 *       | 202 ± 9         | 233 ± 9 *        |
| Average strength (kg)   | 85.8 ± 3.2     | 104.0 ± 3.5 *   | 83.7 ± 3.5      | 102.4 ± 4.2 *    |
| Fasting glucose (mmol/l)| 5.9 ± 0.1      | 5.5 ± 0.1 *     | 9.0 ± 0.4       | 9.0 ± 0.4 #      |
| HbA1c (%)               | 5.8 ± 0.1      | 5.7 ± 0.1 *     | 7.2 ± 0.2       | 7.2 ± 0.2 #      |
| Triacylglycerol (mmol/l)| 1.52 ± 0.13    | 1.49 ± 0.15     | 1.77 ± 0.16     | 1.68 ± 0.14      |

Data are expressed as mean ± SE., #T2D significantly different from control group, *post-training significantly different from pre-training.

Table 2: Substrate kinetics pre- and post training

| Plasma insulin (mU/l) | Pre-training C | Post training C | Pre-training T2D | Post training T2D |
|-----------------------|----------------|-----------------|-----------------|------------------|
| Basal                 | 18.1 ± 2.4     | 16.1 ± 2.1 *    | 16.4 ± 1.2      | 14.6 ± 0.8 *     |
| Clamp                 | 112.5 ± 5.4    | 112.1 ± 5.5     | 107.6 ± 4.8     | 103.1 ± 2.7      |
| Plasma FFA (µmol/l)   | 479.0 ± 22.9   | 454.9 ± 28.3    | 519.4 ± 25.3    | 500.1 ± 34.1     |
| Basal                 | 84.7 ± 7.2     | 67.5 ± 6.9 *    | 107.1 ± 8.7     | 87.6 ± 8.7 *     |
| Clamp                 | 25.8 ± 2.3     | 26.7 ± 2.3      | 18.4 ± 1.4 #    | 21.0 ± 1.4 #     |
| Rd glucose (µmol/kg/min) | 17.1 ± 2.4   | 18.4 ± 2.1      | 6.8 ± 1.4       | 11.1 ± 1.4 #     |
| Basal                 | 8.7 ± 0.7      | 8.3 ± 0.6       | 11.6 ± 0.7 #    | 9.9 ± 0.6 *      |
| Clamp                 | 8.7 ± 0.6      | 8.7 ± 0.6       | 10.3 ± 0.6      | 9.1 ± 0.7        |
| EGP (µmol/kg/min)     | 8.7 ± 0.6      | 8.7 ± 0.6       | 10.3 ± 0.6      | 9.1 ± 0.7        |
| Basal                 | 2.8 ± 0.8      | 1.0 ± 1.0 *     | 2.9 ± 0.5       | 1.4 ± 0.3 *      |
| Clamp                 | -5.7 ± 1.1     | -7.2 ± 1.2      | -7.9 ± 0.6      | -7.7 ± 0.8       |
| CHO oxidation (µmol/kg/min) | 6.5 ± 0.5   | 7.1 ± 0.5       | 8.1 ± 0.6       | 7.3 ± 0.4        |
| Basal                 | 12.5 ± 0.8     | 13.0 ± 0.7      | 11.7 ± 0.8      | 13.2 ± 0.8       |
| Clamp                 | 5.9 ± 0.7      | 5.9 ± 0.6       | 3.6 ± 0.8       | 5.9 ± 0.7 *      |
| NOGD (µmol/kg/min)    | 2.3 ± 0.7      | 1.1 ± 0.6       | 3.5 ± 0.9       | 2.6 ± 0.8        |
| Basal                 | 13.5 ± 1.7     | 13.7 ± 2.3      | 6.7 ± 1.2 #     | 8.0 ± 1.2 #      |
| Clamp                 | 11.3 ± 1.9     | 12.6 ± 1.9      | 3.2 ± 1.4 #     | 5.3 ± 1.2 #      |
| Lipid oxidation (µmol/kg/min) | 1.08 ± 0.05 | 1.03 ± 0.05     | 1.08 ± 0.05     | 1.09 ± 0.05      |
| Basal                 | 0.63 ± 0.04    | 0.55 ± 0.04     | 0.75 ± 0.04 #   | 0.59 ± 0.05 *    |
| Clamp                 | -0.46 ± 0.05   | -0.48 ± 0.06    | -0.32 ± 0.06    | -0.49 ± 0.06 *   |

Data are expressed as mean ± SE., #T2D significantly different from control group, *post-training significantly different from pre-training.
Table 3: Mitochondrial density and UCP3 protein content (AU).

|                  | C                  |                  | T2D                |                  |
|------------------|--------------------|------------------|--------------------|------------------|
|                  | Pre-training       | Post training    | Pre-training       | Post training    |
| Complex I        | 0.61 ± 0.19        | 1.11 ± 0.32      | 0.66 ± 0.18        | 1.65 ± 0.42      |
| Complex II       | 0.61 ± 0.18        | 1.13 ± 0.30      | 0.60 ± 0.15        | 1.74 ± 0.37*     |
| Complex III      | 0.73 ± 0.06        | 1.18 ± 0.12 *    | 0.60 ± 0.05        | 1.55 ± 0.13 # *  |
| Complex IV       | 0.66 ± 0.07        | 1.21 ± 0.09 *    | 0.57 ± 0.07        | 1.63 ± 0.12 # *  |
| Complex V        | 0.76 ± 0.12        | 1.06 ± 0.12 *    | 0.62 ± 0.07        | 1.51 ± 0.14 # *  |
| Average of the complexes | 0.66 ± 0.11        | 1.14 ± 0.16 *    | 0.59 ± 0.08        | 1.62 ± 0.20 *    |
| UCP3             | 0.71 ± 0.12        | 1.43 ± 0.20 *    | 0.39 ± 0.05 #      | 1.50 ± 0.22 *    |
| UCP3 (normalized to mitochondrial density) | 1.22 ± 0.21 | 1.37 ± 0.16 | 0.73 ± 0.09 # | 1.03 ± 0.11 * |

Data are expressed as mean ± SE, #T2D significantly different from control group, *post-training significantly different from pre-training.
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Figure 1
Figure 2

![Graph 2a: Comparison of X vs. Y](image)
P-value: 0.10

![Graph 2b: Comparison of X vs. Y](image)
P-value: 0.07

![Graph 2c: Comparison of X vs. Y](image)

Figure 3

![Graph showing Delta RER](image)
P-value: 0.07

* Significant differences