Physical activity is the key determinant of skeletal muscle mitochondrial function in type 2 diabetes

Physical Activity Is the Key Determinant of Skeletal Muscle Mitochondrial Function in Type 2 Diabetes

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Context: Conflicting data exist on mitochondrial function and physical activity in type 2 diabetes mellitus (T2DM) development.

Objective: The aim was to assess mitochondrial function at different stages during T2DM development in combination with physical exercise in longstanding T2DM patients.

Design and Methods: We performed cross-sectional analysis of skeletal muscle from 12 prediabetic 11 longstanding T2DM male subjects and 12 male controls matched by age and body mass index.

Intervention: One-year intrasubject controlled supervised exercise training intervention was done in longstanding T2DM patients.

Main Outcome Measurements: Extensive *ex vivo* analyses of mitochondrial quality, quantity, and function were collected and combined with global gene expression analysis and *in vivo* ATP production capacity after 1 yr of training.

Results: Mitochondrial density, complex I activity, and the expression of Krebs cycle and oxidative phosphorylation system-related genes were lower in longstanding T2DM subjects but not in prediabetic subjects compared with controls. This indicated a reduced capacity to generate ATP in longstanding T2DM patients only. Gene expression analysis in prediabetic subjects suggested a switch from carbohydrate toward lipid as an energy source. One year of exercise training raised *in vivo* skeletal muscle ATP production capacity by 21 $\pm$ 2% with an increased trend in mitochondrial density and complex I activity. In addition, expression levels of β-oxidation, Krebs cycle, and oxidative phosphorylation system-related genes were higher after exercise training.

Conclusions: Mitochondrial dysfunction is apparent only in inactive longstanding T2DM patients, which suggests that mitochondrial function and insulin resistance do not depend on each other. Prolonged exercise training can, at least partly, reverse the mitochondrial impairments associated with the longstanding diabetic state. **(*J Clin Endocrinol Metab* 97: 3261–3269, 2012)**

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Abbreviations: BMI, Body mass index; CS, citrate synthase, EI, exogenous insulin; HbA_{1c}, glycosylated hemoglobin; IGT, impaired glucose tolerance; IMCL, intramyocellular lipid; MRS, magnetic resonance spectroscopy; mtDNA, mitochondrial DNA; NAD^+, oxidized nicotinamide adenine dinucleotide; NEFA, nonesterified fatty acids; OXPHOS, oxidative phosphorylation system; PCr, phosphocreatine; qPCR, quantitative PCR; T2DM, type 2 diabetes mellitus; VO_{2peak}, peak whole-body oxygen uptake capacity; W_{max}, maximal workload capacity.

Type 2 diabetes mellitus (T2DM) is characterized by peripheral insulin resistance and impairments in pancreatic insulin secretion. Skeletal muscle is the critical organ, accounting for approximately 75% of insulin-mediated glucose disposal (1). The majority of ATP is generated by oxidative phosphorylation in the mitochondria, and alterations in skeletal muscle mitochondrial quality and quantity have been observed in T2DM patients (2, 3). However, other studies have failed to confirm such impairments (4, 5). Similarly, *in vivo* and *ex vivo* measurements of ATP production capacity have been reported to be reduced in skeletal muscle of T2DM patients compared with age- and body mass index (BMI)-matched controls in some (6, 7) but not in all studies (4, 8). These inconsistencies could be (partly) explained by different patient characteristics, lifestyle, and/or analysis methods.

Mitochondrial function is negatively affected by the sedentary lifestyle many T2DM patients have adopted (9). The lack of sufficient physical activity reduces the expression of genes involved in mitochondrial biogenesis and metabolism (10, 11). Reconditioning by endurance- and/or resistance-type exercise training has proven an effective therapeutic strategy in T2DM patients, improving glycemic control, lowering blood pressure, and reducing oxidative stress (12). Physical performance capacity of T2DM patients is strongly associated with their disease status. T2DM patients with complications, such as polyneuropathy, generally display muscle weakness (13), impaired physical performance (9), poor glycemic control,

TABLE 1. Subject characteristics

			T2DM	
	Controls	**Prediabetic**	**Before training**	**After 52 wk training**
n	12	12	8	8
Age (yr)	56 ± 6	58 ± 5	60 ± 7	61 ± 7
BMI (kg/m^2)	32.9 ± 4.6	32.9 ± 6.2	31.7 ± 3.3	31.7 ± 3.9
Body weight (kg)	101.0 ± 14.7	106.5 ± 13.1	96.0 ± 15.2	95.7 ± 16.8
FFM (kg)	70.7 ± 7.1	72.4 ± 6.9	68.3 ± 10.2	68.3 ± 10.2
Truncal fat mass (kg)	15.5 ± 5.5	16.3 ± 2.9	15.4 ± 3.5	16.0 ± 4.1
W_{max} (W)	246.7 ± 42.0	245.5 ± 29.3	150.4 ± 36.7[b,d]	174.6 ± 49.5[e]
W_{max}/kg body weight (W/kg)	2.48 ± 0.5	2.35 ± 0.5	1.61 ± 0.5[b,d]	1.8 ± 0.6[e]
VO_{2peak}/kg body weight (ml/min · kg)	32.3 ± 5.4	33.0 ± 6.3	26.2 ± 3.5[b,d]	26.4 ± 2.3
$VO_{2submax50\%}$	2.07 ± 0.22	2.23 ± 0.27	1.73 ± 0.18[d,f]	1.56 ± 0.25[e]
$VO_{2submax}$/kg body weight (ml/min · kg)	21.3 ± 4.4	21.2 ± 3.4	18.3 ± 2.9	16.8 ± 3.6[e]
O_2-pulse submax	17.7 ± 2.8	18.1 ± 2.6	16.0 ± 1.8[c]	16.1 ± 2.1
Mean arterial pressure (mm Hg)	102 ± 9	103 ± 10	104 ± 8	93 ± 9[f]
HR steady-state submax (beats/min)	118 ± 12	125 ± 11	109 ± 14[c]	98 ± 13[f]
HR_{max} (beats/min)	166 ± 13	172 ± 11	135 ± 25[b,d]	131 ± 25
% predicted HR_{max}	99 ± 8	103 ± 7	81 ± 14[b,d]	80 ± 14
Activity level (MET h/d)	19.3 ± 7.4	19.7 ± 8.4	11.3 ± 4.8[a,c]	12.5 ± 4.1
Fasting glucose (mM)	5.7 ± 0.2	6.5 ± 0.5[b]	9.4 ± 2.8[b,d]	10.8 ± 3.7
Fasting insulin (mU/ml)	16.2 ± 9.1	21.5 ± 9.5	ND	ND
HOMA index	4.1 ± 2.2	6.3 ± 2.7[a]	ND	ND
2-h glucose (mM)	5.7 ± 1.3	8.4 ± 2.5[a]	ND	ND
2-h insulin (mU/ml)	74.8 ± 40.5	140.2 ± 65[b]	ND	ND
HbA_{1c} (%)	5.3 ± 0.3	5.5 ± 0.2	7.4 ± 0.8[b,d]	7.4 ± 0.8
NEFA (mM)	0.31 ± 0.10	0.40 ± 0.12	0.45 ± 0.23[a]	0.57 ± 0.25
Time with T2DM (yr)	NA	NA	12.5 ± 7.7	13.5 ± 7.7
Time with insulin therapy (yr)	NA	NA	8 ± 9	9 ± 9
Total EI (IU)	NA	NA	92.6 ± 37.0	92.0 ± 28.1
EI/kg (IU)	NA	NA	0.98 ± 0.40	0.98 ± 0.38

Characteristics for T2DM subjects are calculated for the eight subjects who completed the 52 wk training to allow comparison before and after training. For cross-sectional comparison with prediabetic and control group, 10 T2DM subjects were analyzed, but the eight training subjects are representative for the whole group (n = 10) and did not affect statistical analyses of differences when compared with the prediabetic or control group. $VO_{2\ submax50\%}$ is oxygen uptake at 50% of VO_{2peak} capacity; HR_{max} is maximal heart rate during the VO_{2peak} test; percent predicted HR_{max} is the percentage of the age-predicted HR_{max}; HR steady-state submax is the steady-state heart rate during the submaximal test; 2-h glucose/insulin is the glucose/insulin concentration 2 h after ingestion of a glucose load during the oral glucose tolerance test. FFM, Fat-free mass; HOMA, homeostasis model assessment; HR, heart rate; IU, insulin unit; MET, metabolic equivalents; NA, not applicable; ND, not determined; O_2-pulse submax (ml/HR), VO_2/heart rate.

[a,b] Significantly different from controls: [a] $P < 0.05$; [b] $P < 0.01$.

[c,d] Significantly different from prediabetic: [c] $P < 0.05$; [d] $P < 0.01$.

[e,f] Significantly different from T2DM before training with paired *t* test: [e] $P < 0.05$; [f] $P < 0.01$.

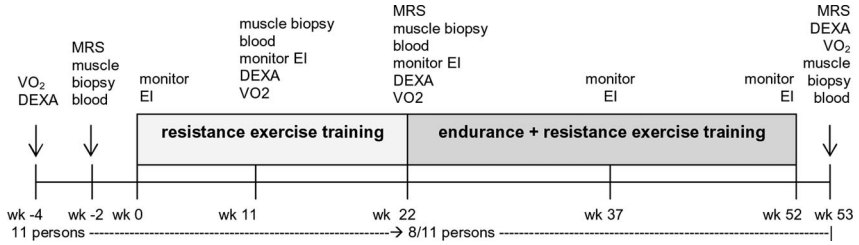

FIG. 1. Overview of the study protocol, including time points of sample/data collection: VO_2, oxygen uptake at peak and 50%; MRS, ^{31}P and ^{1}H MRS measurements of mitochondrial function and IMCL content, respectively; monitor EI requirement; DEXA, dual-energy x-ray absorptiometry to assess body composition and fat-free mass.

and a high cardiovascular risk profile (14). These subjects are generally not advised to participate in more intense exercise intervention programs because insufficient data are available on the clinical benefits and potential health risks of such interventions. Nevertheless, prolonged resistance- and interval-type exercise training has been shown to augment maximal workload capacity, reduce resting blood pressure, and attenuate the progressive increase in exogenous insulin (EI) requirements in longstanding, insulin-treated T2DM patients with comorbidities (15).

Our study analyzed mitochondrial quality and quantity and global gene expression profiles in skeletal muscle from prediabetic and longstanding T2DM patients compared with normoglycemic age- and BMI-matched controls. Furthermore, we determined the capacity of prolonged exercise training as a means to attenuate or even reverse the progression of the T2DM disease in longstanding insulin-treated T2DM patients.

Subjects and Methods

Prediabetic, T2DM, and control subjects

The Human Investigation Review Committee of the Máxima Medical Center (Veldhoven, The Netherlands) approved the study protocol. Eleven patients with longstanding (>5 yr) T2DM treated with EI (>2 yr) and 12 impaired glucose tolerant (IGT) prediabetic and newly identified T2DM subjects were recruited and age and BMI matched to 12 normoglycemic controls (Table 1). Subjects were all Caucasian males except one male control subject of Mexican-Surinam origin, and all provided written informed consent. Control and prediabetic subjects had no family history of T2DM and were selected based on an oral glucose tolerance test according to the World Health Organization criteria (16). Control subjects showed normal fasting glucose concentrations and glucose tolerance. The prediabetic group consisted of five subjects with elevated fasting plasma glucose concentration (impaired fasting glucose), three showed IGT, and four were recently diagnosed with T2DM (for criteria see Supplemental Methods, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). None of the subjects in the prediabetic group were using any glucose-lowering medication, and all showed glycosylated hemoglobin (HbA_{1c}) levels below 6.0%. All T2DM subjects were on EI treatment and had been on a stable medication regimen over the last 3 months before being recruited. See Supplemental Methods for complete medication list. Subjects did not have the diabetogenic m.3243A→G mutation, impaired liver function, renal failure, severe retinopathy, or a history of severe cardiovascular problems.

Body composition, blood pressure, and physical performance measures

Body mass, waist circumference, segmental and whole-body bone and fat-free mass, systolic and diastolic blood pressure, peak whole-body oxygen uptake capacity (VO_{2peak}), maximal workload capacity (W_{max}), habitual physical activity level, and quality of life were assessed as described before (8, 15).

Training procedures, adverse events, and *in vivo* ^{31}P magnetic resonance spectroscopy (MRS) analysis

All T2DM patients followed the same 1-yr supervised exercise protocol as shown in Fig. 1 and described in more detail in Supplemental Methods. Eight of the 11 longstanding T2DM patients completed the 52-wk exercise intervention. Three subjects stopped after 5 months, because of psychosocial (2) or medical reasons (1). *In vivo* analysis of ^{31}P MRS phosphocreatine (PCr) recovery rate and ^{1}H MRS intramyocellular lipid (IMCL) content were performed 2 wk before the start and 1 wk after completing the training program. ^{31}P and ^{1}H MRS measurements and subsequent data analysis were performed (8). Because of technical difficulties, ^{31}P MRS and ^{1}H MRS IMCL data from one T2DM patient were excluded from analysis.

Blood analysis and *ex vivo* mitochondrial analysis

In the evening, all subjects received the same standardized meal (mean ± SD 41.2 ± 15.2 kJ/kg body weight, containing 39.5% energy from fat, 15.1% energy from protein, and 45.4% energy from carbohydrate), took their medication, and then remained fasted. The next morning, a venous blood sample was collected after 5–10 min of supine rest. Fasting plasma glucose, nonesterified fatty acids (NEFA), serum cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triacylglycerol, blood HbA_{1c}, plasma insulin, serum adiponectin, C-reactive protein, and C-peptide were analyzed as described previously (15). A percutaneous muscle biopsy was performed from the vastus lateralis muscle, freed from any visible nonmuscle material, immediately frozen in liquid nitrogen, and stored at −80 C. Muscle homogenates were prepared from frozen muscle, and citrate synthase (CS), complex I, and complex IV were measured (17, 18) and analyzed. Detailed description of the analysis is explained in Supplemental Methods. DNA was isolated using the Wizard Genomic DNA isolation kit (Promega, Madison, WI). The mitochondrial DNA (mtDNA) copy number quantification, screening for mitochondrial deletions, and detection of the m.3243A→G mutation were performed using (real-time) PCR as described in Supplemental Methods.

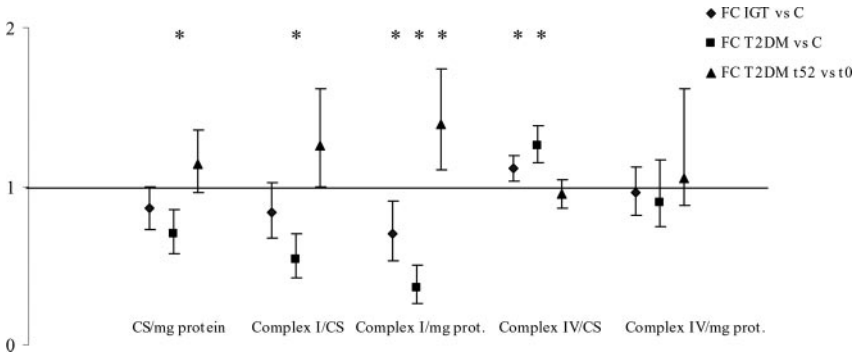

FIG. 2. Alterations in CS and OXPHOS complex I and IV activity. Fold change (FC) in CS, complex I and complex IV activity in prediabetic (IGT) (◆) and long-term T2DM (■) compared with controls (C), and after 52 wk training in the T2DM group (T2DM t52) (▲) compared with T2DM at time zero (t0). Data are expressed as mean fold change (± confidence interval). The Akaike information criterion was used for statistical modeling. *, Significantly changed.

Gene expression analysis and real-time PCR validation

Total RNA was isolated from muscle using the TRIzol reagent (Invitrogen, Carlsbad, CA) and purified with the RNeasy clean-up kit (QIAGEN, Hilden, Germany). Muscle RNA was amplified and hybridized on Affymetrix (Santa Clara, CA) U133 plus 2.0 arrays. The microarray data reported in this manuscript have been deposited in the NCBI Gene Expression Omnibus (GEO), accession number GSE19420. Multivariate Gaussian linear regression was subsequently used for statistical analysis followed by GenMAPP/MAPPFinder and DAVID for pathway and process analysis of significantly changed genes (for full description, see Supplemental Methods).

Real-time quantitative PCR (qPCR) was used for validation. Primers (Supplemental Table 1) were designed using Primer Express software version 3.0 (Applied Biosystems, Foster City, CA). The qPCR was performed as described (19). The mRNA levels were normalized to the TATA-box binding protein (*TBP*) housekeeping gene, and results were analyzed in the same way as the microarrays (Supplemental Methods).

Statistical analysis EI requirements

The quantity of EI requirements were analyzed using a multivariate Gaussian linear regression including time and a first-order autocorrelation and/or a random effect to take into account the dependence among the time series of observations from the same subject. The Akaike information criterion was used to assess whether there was a linear time trend both during the exercise-training and nontraining period of insulin intake (detailed description in Supplemental Methods).

Results

Physical and *ex vivo* mitochondrial characteristics of prediabetic and T2DM patients

Oral glucose tolerance tests were performed to assess whole-body glucose tolerance as a surrogate marker for insulin sensitivity. Homeostasis model assessment index and fasting and 2-h glucose and insulin levels were significantly elevated in prediabetic subjects when compared with the normoglycemic controls (Table 1). In the T2DM patients at time zero, VO_{2peak}, W_{max}, maximal heart rate, and activity level (MET hours per day) were significantly lower than in the other two groups. In addition, NEFA content and fasting glucose levels were higher when compared with controls and prediabetic subjects. Mitochondrial density analyzed by CS activity and complex I per CS were significantly reduced in the T2DM patients, whereas complex IV per CS was slightly elevated in both prediabetic and T2DM subjects (Fig. 2). The mtDNA copy number was comparable for control, prediabetic, and T2DM groups [3201 ± 719, 3598 ± 742, and 3607 ± 622 (mean ± SD), respectively]. Six of the 12 controls had no mtDNA deletion, three had a single deletion, and three had multiple deletions. In the 12 prediabetic subjects, no deletion was detectable in half of the group, four subjects had a single deletion, and two subjects had multiple deletions. Finally, in the longstanding T2DM subjects, five of nine had no deletion, one had a single deletion, and three had multiple deletions.

Global gene expression analysis in muscle of prediabetic and T2DM patients

A total of 1707 genes were differentially expressed with a fold change over 10% (Supplemental Table 4). A total of 153 genes were differently expressed in both the prediabetic and T2DM group compared with controls, 550 specifically in the prediabetic subjects and 851 in the T2DM patients. Expression of the adipocyte-specific gene adiponectin did not pass the background intensity threshold, indicating no significant contamination of adipocytes in the muscle specimen. In the prediabetic group, an equal number of genes were up- and down-regulated, but in the T2DM group, the majority (70%) was lower (Supplemental Fig. 1). Fold changes were in general small. In the prediabetic group, the largest fold changes were 0.57 and 1.93, in the T2DM group 0.43 and 1.91. The qPCR analysis of 11 genes showed a significant fold change in the same direction for eight genes and a nonsignificant trend for the remaining three (Supplemental Table 1). Differently expressed genes were used for gene ontology analysis (GenMAPP/MAPPFinder program) to identify altered pathways (Supplemental Table 5). Next, we used DAVID for gene ontology based analysis of biological processes (Supplemental Tables 7–9). In general, DAVID confirmed the MAPPFinder processes, although adding processes not included in the local MAPP

of MAPPFinder, *e.g.* chromatin modifiers, oxidized nicotinamide adenine dinucleotide (NAD^+)/reduced NAD recycling, tRNA synthesis, amino acetylation, and amino acid synthesis.

Processes altered in prediabetic subjects

In the prediabetic group, expression of key genes involved in glycolysis were decreased compared with controls. In contrast, genes regulating fatty acid β-oxidation and fatty acid and triglyceride synthesis were expressed to a greater extent, suggesting at the RNA level increased use of fatty acids as an energy source (Fig. 3 and Supplemental Table 5). Genes of the striated muscle contraction pathway encoding components of type 2 or oxidative fibers were up-regulated in prediabetic subjects, whereas expression of type 2 glycolytic muscle fiber components was lower.

Processes altered in longstanding T2DM subjects

In longstanding T2DM subjects, expression was decreased of genes involved in substrate transport into mitochondria, conversion of pyruvate into acetyl-coenzyme A, and the aspartate-malate shuttle that recycles reduced NAD/NAD^+ used for ATP synthesis in the mitochondria. In addition, the ability to use glucose as an energy source and the expression of genes involved in Krebs cycle and electron transport chain were lower in T2DM patients (Fig. 3). In addition, ketone body synthesis was triggered at the RNA level as a result of up-regulation of *HMGCS2* and decreased expression of key regulators of ketone body metabolism *OXCT1* and *BDH* in T2DM patients. Another process altered in the T2DM group (Supplemental Table 5) was circadian rhythm, of which components are regulated in a time- and exercise-dependent manner and play a role in various processes, including transcription, cell-cycle regulation, and glucose uptake (20). In addition, the expression of a number of genes was altered in the adipogenesis pathway, which contains genes involved not only in the transcriptional regulation of adipogenesis and cell-cycle regulation but also in lipid storage and energy metabolism.

Increased physical performance and mitochondrial capacity after 52 wk of exercise training in T2DM patients

Eight longstanding T2DM subjects completed a 52-wk exercise training protocol (Fig. 1). They participated on

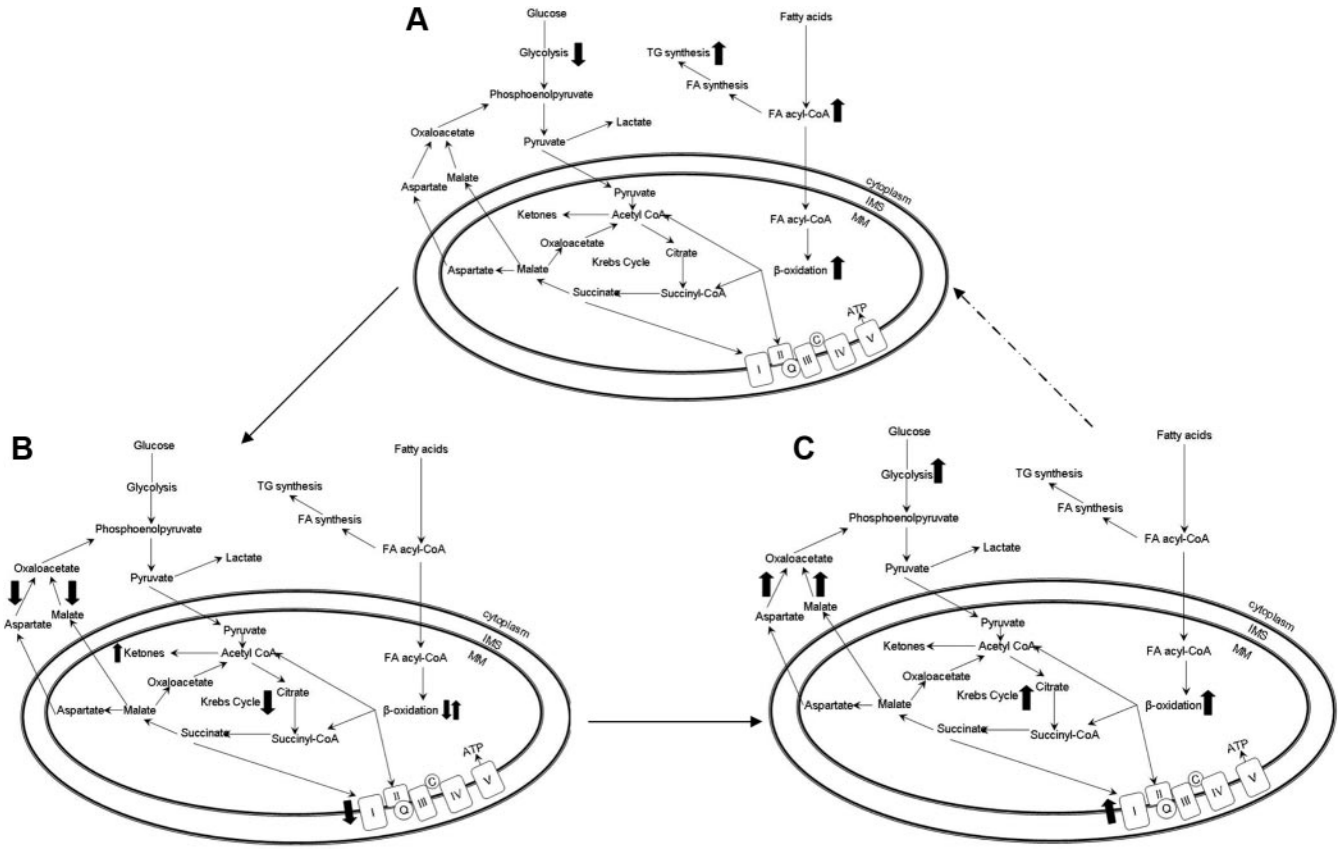

FIG. 3. Schematic overview of the most significant gene expression changes in energy metabolism per group. Gene expression alterations in energy metabolism of prediabetic (A), longstanding T2DM subjects (B) compared with controls, and the effects of 52 wk exercise training in T2DM subjects (C). *Upward arrows* indicate stimulation and *downward arrows* indicate decrement of a process. Genes or processes without arrows had no altered expression pattern. IMM, Inner mitochondrial matrix; IMS, intermembrane space.

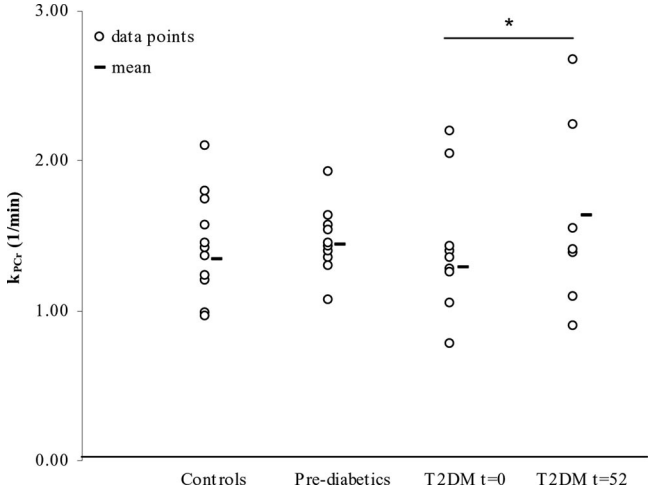

FIG. 4. The k_{PCr} activity in controls, prediabetics, and T2DM before (t=0) and after 52 wk exercise training (t=52) The Akaike information criterion was used for statistical modeling. *, Significantly changed in T2DM group at 52 wk compared with T2DM at time zero, analyzed with paired *t* test, $P < 0.05$.

average in 1.9 ± 0.4 (mean ± SD) exercise sessions per week, representing 62 ± 13% of all sessions. After 52 wk, W_{max}, VO_{2peak}, and heart rate improved significantly compared with baseline measurements (Table 1). *In vivo* ^{31}P MRS measurements showed that exercise training significantly increased the PCr recovery rate constant [21 ± 2% (mean ± SD)], which is a measure for oxidative capacity (Fig. 4 and Supplemental Table 2). ^{1}H MRS measurements showed no changes in IMCL content. As shown in Figure 2, *ex vivo* analysis indicated altered mitochondrial density and complex I and IV activity after 52 wk exercise training. No significant differences between time zero and 52 wk in mtDNA content [3662 ± 641 and 3513 ± 1023 (mean ± SD), respectively], large-scale mtDNA deletions, or quality of life [61 ± 24 and 65 ± 23 (mean ± SD)] on the RAND 36-item Health Survery scale of 0–100 were observed. In addition, glycemic control was similar before and after training, but the EI requirements were significantly reduced after 52 wk training when compared with the expectations derived from the 3 preceding years (+0.80 U EI/month) (Fig. 5).

The effect of 52 wk exercise training on skeletal muscle gene expression in T2DM patients

After 52 wk training, 1095 genes were altered in longstanding T2DM subjects; 387 were decreased, and 708 increased (Supplemental Table 4). The largest fold changes were 0.59 and 3.58. Some altered processes were the same as in prediabetic/T2DM patients compared with controls, but others were specific for exercise training (Supplemental Tables 5 and 9). Exercise training significantly enhanced expression of a number of genes involved in energy metabolism, like electron-transport chain, Krebs cycle, fatty acid β-oxidation, and glycolysis (Fig. 3 and Supplemental Table 6). Similarly, the striated muscle contraction pathway, which includes genes that constitute muscle fibers involved in the force-generating process of contraction, was significantly altered not only after training but also in untrained longstanding T2DM subjects compared with controls. In the untrained longstanding T2DM patients, the differences in gene expression were most likely attributed to lower physical activity and muscle wasting in the diabetes patients. In contrast, prolonged exercise training altered the expression profile toward the expression profile observed in the prediabetic subjects. In line with this, prolonged exercise training also increased the epidermal growth factor receptor type 1 and heme biosynthesis pathways, both of which are involved in a wide range of cellular processes, like differentiation, proliferation, and transcription/translation cell-cycle regulation. In contrast, the catabolic proteasome degradation pathway was decreased after training.

Discussion

Despite extensive investigations, the role of mitochondrial dysfunction in T2DM development is still under debate. In this study, we performed a cross-sectional comparison of prediabetic subjects, longstanding insulin-treated T2DM patients, and age- and BMI-matched controls, which allowed us to analyze mitochondrial function and integrity at different stages in diabetes development. Although *in vivo* mitochondrial function (Fig. 4) (8), mtDNA density, and mitochondrial integrity did not differ between groups, *ex vivo* CS activity and complex I activity per mitochondrion were significantly reduced in the T2DM group. The changes observed in mitochondrial capacity of long-term T2DM patients were supported by reduced expression levels of genes involved in the Krebs cycle and electron transport chain. In contrast to the long-term diagnosed T2DM patients, mitochondrial density and activity were not significantly different in prediabetic subjects when compared with the age- and BMI-matched control group. Furthermore, gene expression analysis did not identify altered mitochondrial metabolism but only suggested increased expression of genes involved in use of fatty acids as an energy source.

Our results support the hypothesis of Lanza and Nair (21) that mitochondrial function is not related to insulin sensitivity and that the joint observation of mitochondrial dysfunction and insulin resistance is coincidental or linked by a common factor. In line with this, no correlation between ATP_{max} and insulin sensitivity within a group of T2DM patients was found in the study of Bajpeyi *et al.*

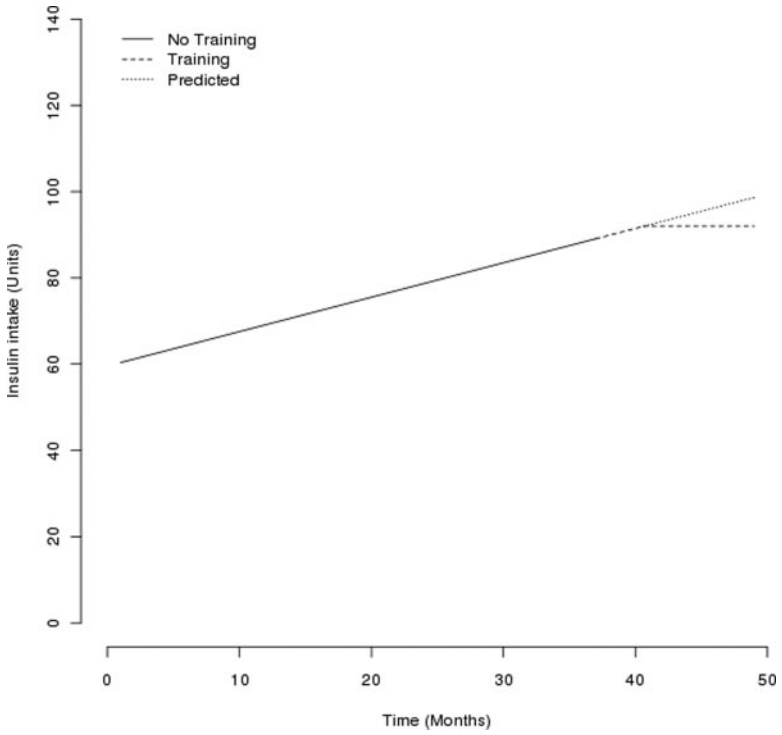

FIG. 5. EI requirements of T2DM patients before and during the exercise training period. The *solid line* represents the mean EI requirements of the eight longstanding T2DM subjects before participating in the exercise training study, and the EI requirements during the 52 wk of exercise training, from months 36–48, indicated by the *dashed line*. The *dotted line* represents the expected increase in EI requirement without exercise training, which was calculated based on the EI increase before participation in the training protocol.

(22). Absence of reduced PCr recovery rate or CS or complex I levels in the prediabetic/newly identified T2DM subjects compared with matched controls in our study and others (4, 22, 23) suggests that mitochondrial dysfunction is not a prerequisite for the development of insulin resistance and/or type 2 diabetes. In contrast, a few studies have observed an *ex vivo* reduced mitochondrial capacity when compared with age- and BMI-matched controls and corrected for mitochondrial density (6, 24). The conflicting data presented in the literature and the lack of consistency in results regarding mitochondrial capacity in T2DM are partly the result of the different outcome parameters analyzed and differences in subject selection criteria. The tight relation between physical activity and mitochondrial function requires matching for VO_{2max} in addition to age and BMI. Lack of matching (24) or inclusion of subjects with a broad range in VO_{2max} values (6) may largely influence the outcome. Exercise training and detraining studies also emphasize the tight connection between mitochondrial capacity and physical activity. This is reflected by a 70% increase in mitochondrial CS after 6 wk endurance training, whereas a 12–28% reduction in ATP production was observed 3 wk after ending the training (25, 26). These data support the hypothesis that impaired mitochondrial function is more likely the result of a reduced ATP demand due to a lower physical activity level (11) and explains the lack of a significant correlation between ATP_{max} and insulin sensitivity (22). In addition, mitochondrial function may be affected by persisting postprandial hyperglycemia despite exogenous insulin therapy, which is known to improve mitochondrial function (5). Furthermore, the observed reduction in basal ATP production in insulin-resistant offspring of T2DM patients (27) may also be the result of impaired insulin signaling affecting insulin-dependent mitochondrial process (28, 29), which is also observed in studies analyzing the effect of insulin infusion on mitochondrial ATP production (5, 30). The unique strengths of our study are the cross-sectional *ex vivo* and *in vivo* analysis of the same individual, and selection of two groups at different stages of T2DM, namely a prediabetic group, which was never treated with oral glucose-lowering medication and were carefully matched for VO_{2max}, and a second group of longstanding T2DM patients treated with exogenous insulin. Both *ex vivo* and *in vivo* analysis were performed in these groups. Furthermore, analysis of the group of insulin-treated T2DM patients provided insights in the effect of long-term T2DM with associated complications and reduced physical activity level on mitochondrial capacity.

Taken together, the lack of a consistent reduction in oxidative capacity indicates that mitochondrial dysfunction is not a prerequisite for T2DM development. Additional evidence for this hypothesis is provided by the disproportionately large capacity for mitochondrial ATP generation (31), the fact that mice on a high-fat diet develop insulin resistance despite an increased mitochondrial content (32), that normal mitochondrial density and function is observed in insulin-resistant women with polycystic ovary syndrome (33), and the prevalence of enhanced mitochondrial function in diabetic Asian Indians (34). Furthermore, mitochondrial respiratory chain defects that result in severely decreased ATP production in skeletal muscle tissue are generally not accompanied by T2DM development. One exception is the mtDNA m.3243A→G mutation, but development of diabetes in these subjects is not associated with a further reduction in *in vivo* PCr recovery rate (35).

Although exercise training has only a limited impact on insulin sensitivity (28), it is regarded as an effective strategy to treat chronic metabolic disease (36). However, longstanding insulin-treated T2DM patients with reduced

muscle strength and a lower activity level are often unable to participate in more generic endurance exercise training programs. A combined resistance- and interval-type exercise training program of 10 and 22 wk has been proven feasible and beneficial for this subgroup of T2DM patients (15, 37). To study the long-term effects, eight longstanding T2DM patients completed a 1-yr training protocol. This intervention resulted in increased muscle strength, greater submaximal aerobic capacity, and improved *in vivo* and *ex vivo* mitochondrial functioning. Gene expression analysis was used as an indicator of the altered processes and suggested that fatty acid β-oxidation, Krebs cycle, and oxidative phosphorylation system (OXPHOS)-related genes were induced. These processes are generally stimulated by exercise training (38) and were specifically decreased in the T2DM subjects compared with controls. This indicated that the right processes were targeted, but analysis on the protein level is required to reach a definite conclusion. Training also improved blood lipid profiles of T2DM subjects (Supplemental Table 3), because high-density lipoprotein and low-density lipoprotein cholesterol levels were, respectively, significantly increased and decreased after 1 yr of exercise training. Glycemic control was not changed with respect to changes in fasting glucose concentration or HbA_{1c} levels. However, their exogenous insulin requirements stabilized in contrast to the increasing requirements in the 3 preceding years (Fig. 5). Because we did not observe changes in C-peptide, the stabilization of exogenous insulin requirements can be attributed to the adaptive response to training on whole-body insulin sensitivity, *e.g.* by decreasing hepatic glucose output, increasing muscle mass, increasing glycogen and im lipid storage capacity, and improving skeletal muscle perfusion. In line with our findings, a 12-wk training intervention of well-controlled metformin-treated T2DM subjects also showed that exercise training restored metabolic flexibility and increased muscle strength and mitochondrial density and activity but did not affect fasting glucose concentration or HbA_{1c} levels (39). Finally, gene expression analysis indicated reduced muscle wasting and increased muscle regeneration, especially for type I muscle fibers, which possibly contributes to increased fatty acid oxidation. Exercise training may, therefore, reverse the loss of oxidative fibers in metabolic syndrome and T2DM patients (40).

Despite the application of whole-body and *ex vivo* measurements in various T2DM subpopulations and analyzing the impact of prolonged exercise training, the present study also has its limitations. First of all is the mixture of IGT, impaired fasting glucose, and newly identified T2DM patients in the prediabetic group, because minor differences may exist between these subgroups. Second is the lack of whole-body insulin sensitivity data measured by a hyperinsulinemic-euglycemic clamp. Third, in our *ex vivo* analysis, we assessed parameters to determine OXPHOS capacity, but not OXPHOS effectiveness, *e.g.* reactive oxygen species production, proton leakage, and/or mitochondrial size.

In summary, our most important findings in skeletal muscle of prediabetic and insulin-treated T2DM subjects are that 1) mitochondrial dysfunction is apparent only in inactive longstanding T2DM patients, which indicates that mitochondrial function and insulin resistance do not depend on each other, and 2) mitochondrial dysfunction in longstanding T2DM patients using exogenous insulin therapy can, at least partly, be reversed by prolonged endurance- and resistance-type exercise training.

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