Exercise Response Variations in Skeletal Muscle PCr Recovery Rate and Insulin Sensitivity Relate to Muscle Epigenomic Profiles in Individuals With Type 2 Diabetes

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Diabetes Care 2018;41:2245–2254 | https://doi.org/10.2337/dc18-0296

OBJECTIVE

Some individuals with type 2 diabetes do not reap metabolic benefits from exercise training, yet the underlying mechanisms of training response variation are largely unexplored. We classified individuals with type 2 diabetes (n = 17) as nonresponders (n = 6) or responders (n = 11) based on changes in phosphocreatine (PCr) recovery rate after 10 weeks of aerobic training. We aimed to determine whether the training response variation in PCr recovery rate was marked by distinct epigenomic profiles in muscle prior to training.

RESEARCH DESIGN AND METHODS

PCr recovery rate as an indicator of in vivo muscle mitochondrial function in vastus lateralis (^{31}P-magnetic resonance spectroscopy), insulin sensitivity (M-value; hyperinsulinemic-euglycemic clamp), aerobic capacity (Vo_{2peak}), and blood profiles were determined pretraining and post-training. Muscle biopsies were performed pretraining in vastus lateralis for the isolation of primary skeletal muscle cells (HSkMCs) and assessments of global DNA methylation and RNA sequencing in muscle tissue and HSkMCs.

RESULTS

By design, nonresponders decreased and responders increased PCr recovery rate with training. In nonresponders, insulin sensitivity did not improve and glycemic control (HbA_{1c}) worsened. In responders, insulin sensitivity improved. Vo_{2peak} improved by ∼12% in both groups. Nonresponders and responders were distinguished by distinct pretraining molecular (DNA methylation, RNA expression) patterns in muscle tissue, as well as in HSkMCs. Enrichment analyses identified elevations in glutathione regulation, insulin signaling, and mitochondrial metabolism in nonresponders pretraining, which was reflected in vivo by higher pretraining PCr recovery rate and insulin sensitivity in these same individuals.

CONCLUSIONS

A training response variation for clinical risk factors in individuals with type 2 diabetes is reflected by distinct basal myocellular epigenomic profiles in muscle tissue, some of which are maintained in HSkMCs, suggesting a cell-autonomous underpinning. Our data provide new evidence to potentially shift the diabetes treatment paradigm for individuals who do not benefit from training, such that supplemental treatment can be designed.

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Received 8 February 2018 and accepted 15 July 2018.

Clinical trial reg. no. NCT01911104, clinicaltrials.gov.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc18-0296/-/DC1.

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By 2050, the number of people who have received a diagnosis of diabetes in the U.S. will reach 29 million (1). Skeletal muscle insulin resistance is a hallmark of type 2 diabetes and is linked with deficient skeletal muscle (muscle) mitochondrial oxidative capacity (function) (2,3). Considering group averages, exercise training restores muscle mitochondrial function in individuals with type 2 diabetes to levels observed in healthy individuals, in parallel with improvements in insulin sensitivity (4). Intervention studies (5–7) have shown that glucose homeostasis, insulin sensitivity, or muscle mitochondrial density fails to improve with supervised exercise training in ~20–40% of individuals. Conclusions about the beneficial effects of exercise training are too often drawn based on average responses, and little is known about those individuals who do not respond favorably to training.

Variation in the training response in vivo has been linked with distinct basal genetic and transcriptional profiles in muscle tissue (8–10); however, the influence of the epigenome on training response variation in individuals with type 2 diabetes remains poorly understood. Individuals within a family respond more similarly than those of different families, suggesting that DNA sequence variation or epigenomic modifications contribute to training response variability (6,11,12). For example, in first-degree relatives of patients with type 2 diabetes, the single nucleotide polymorphism rs540467 in the *NDUF6B* gene was linked to exercise response variation in muscle ATP synthesis rates in vivo after acute aerobic bouts (8). In individuals at risk for the development of type 2 diabetes, higher activation of transforming growth factor-β (*TGFB1*) signaling contributed to a blunted exercise-induced improvement in insulin sensitivity via the suppression of key regulators of muscle mitochondrial fuel oxidation (13). Moreover, DNA hypomethylation is reported to be an early event in contraction-induced gene activation (transcriptional upregulation) in muscle, and chronic exercise training modifies genome-wide DNA methylation patterns in human muscle, specifically in genes related with metabolic processes and mitochondrial function (e.g., *PPARGC1A*, *PDK4*, *PPARD*) (14). We have previously reported (10) large differences in pretraining mRNA expressions of genes involved in substrate metabolism and mitochondrial biogenesis in muscle tissue of individuals with type 2 diabetes who did or did not improve their metabolic profile with exercise training, suggesting a unique baseline gene expression pattern, which may underlie an individual's lack of training response.

In this study, we classified individuals with type 2 diabetes as nonresponders or responders, based on their changes in phosphocreatine (PCr) recovery rate after 10 weeks of supervised aerobic training. Training responses in metabolic risk factors and cardiorespiratory fitness were compared between groups. Global levels of pretraining DNA methylation and RNA expression were analyzed in muscle tissue and in primary human skeletal muscle cells (HSkMCs) of nonresponders and responders. We hypothesized that training response variation in muscle mitochondrial function in vivo would be marked by distinct epigenomic profiles in the muscle prior to the exercise training intervention.

RESEARCH DESIGN AND METHODS

Participants

Seventeen individuals with type 2 diabetes (n = 8 females; mean age 50.7 ± 1.9 years; BMI 34.9 ± 0.9 kg/m^2) completed the study. Six individuals dropped out of the study for personal reasons and/or at the discretion of the Principal Investigator. Participants were sedentary, defined as not being physically active ≥3 days per week for the previous 6 months. Type 2 diabetes was determined by self-report and/or fasting plasma glucose ≥7.0 mmol/L. HbA_{1c} levels at the time of study enrollment had to be ≤8.5% (69 mmol/mol) for individuals with type 2 diabetes receiving glucose-lowering medication (e.g., metformin, sulfonylurea) and between 6.0% (42 mmol/mol) and 8.5% (69 mmol/mol) for individuals with type 2 diabetes being treated with diet alone. Participants ceased glucose-lowering treatment 15–17 days prior to the start of the aerobic training program and remained off medication for the entire duration of the study. Two individuals resumed treatment with their prescribed sulfonylurea during the study because of consistent hyperglycemia (fasting plasma glucose >22.2 mmol/L). Participants were asked to maintain dietary behavior during the study, and a 3-day food recall was collected pretraining and post-training. The 2 days prior to the pretraining and post-training metabolic assessments, participants were provided meals as part of a standard American diet (35% fat, 16% protein, 49% carbohydrate) and instructed to consume all of the food provided. On the evening prior to the hyperinsulinemic-euglycemic clamp, participants stayed overnight in the metabolic ward and consumed a meal as part of a standard American diet. All participants provided written informed consent. All procedures were performed under a research protocol approved by the Florida Hospital Institutional Review Board.

Aerobic Training Protocol

Participants underwent supervised aerobic training on a treadmill for 10 weeks (4 days/week) of a ramped training protocol proven to improve insulin sensitivity in individuals with overweight and obesity (15,16). All exercise sessions were supervised to ensure that the targeted intensity (based on target heart rate) and duration were achieved. During weeks 1–4, participants performed exercise for at least 20 min per session at an intensity of 50–70% $V_{O_{2peak}}$. During weeks 5–8, participants increased the exercise time to 45 min per session at the same intensity. During weeks 9–10, participants performed exercise for 45 min per session at 75% $V_{O_{2peak}}$.

In Vivo Muscle Mitochondrial Function

In vivo muscle mitochondrial function was evaluated by PCr recovery rate in vastus lateralis using phosphorus (^{31}P) magnetic resonance spectroscopy on a 3-T Achieva magnet (Philips Healthcare, Andover, MA), as previously described (2). The PCr time constant (tau) was used to measure PCr recovery rate (1/tau). The coefficient of variation for this measurement was 4.5%. Participants were separated into two groups (nonresponders, n = 6; responders, n = 11) based on the percentage change in muscle mitochondrial function in vivo after 10 weeks of aerobic training, which was defined as the PCr recovery rate post-training minus the PCr recovery rate pretraining, divided by the PCr recovery rate pretraining. Participants who decreased the PCr recovery rate were identified as nonresponders; participants who increased the PCr recovery rate were identified as responders (Fig. 1*A*).

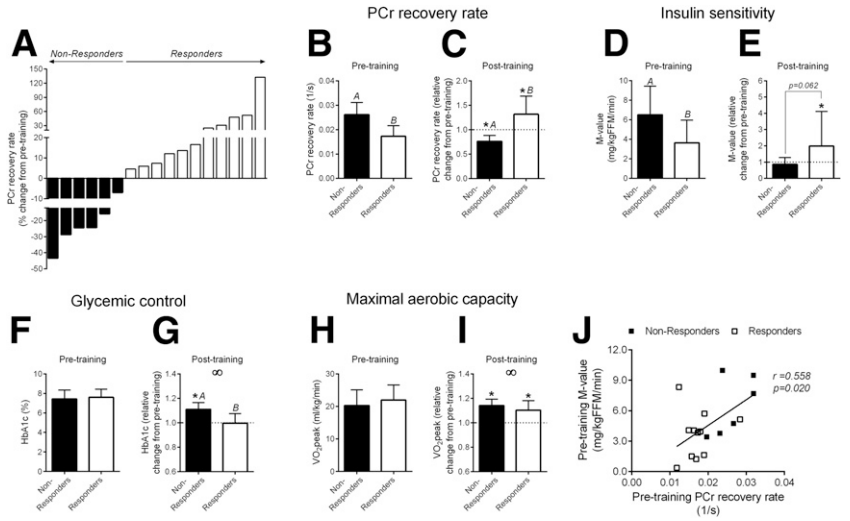

Figure 1—Pretraining values and training-induced changes in metabolic risk factors and cardiorespiratory fitness. *A*: The individual percentage changes in PCr recovery rate in vivo in skeletal muscle with training. Training response variation for PCr recovery rate was used to classify individuals with type 2 diabetes as nonresponders (n = 6) or responders (n = 11) after 10 weeks of aerobic training. Group averages in nonresponders and responders for PCr recovery rate pretraining (*B*), training-induced change in PCr recovery rate (*C*), insulin sensitivity pretraining (*D*), training-induced change in insulin sensitivity (*E*), glycemic control (HbA_{1c}) pretraining (*F*), training-induced change in glycemic control (HbA_{1c}) (*G*), $V_{O_{2peak}}$ (cardiorespiratory fitness) pretraining (*H*), and training-induced changes in $V_{O_{2peak}}$ (cardiorespiratory fitness) (*I*). *J*: Correlation between pretraining PCr recovery rate and pretraining M-value. $^*P < 0.050$ compared with pretraining within the same group, $^\infty P < 0.050$ ANOVA main time effect (pretraining vs. posttraining). Different capital letters [A and B] indicate significant differences between groups posttraining. Data are mean ± SD. FFM, fat-free mass.

Insulin Sensitivity

Insulin sensitivity was measured by hyperinsulinemic-euglycemic clamp, as previously described (17) with modifications. Participants arrived at the research facility the evening prior to the hyperinsulinemic-euglycemic clamp, consumed a standard American meal, and stayed overnight in the metabolic ward. After an overnight fast, insulin (100 mU/m^2/min) and 20% glucose were administered for 2 h to maintain the plasma glucose concentration at ~5.0 mmol/L. Plasma levels of glucose were measured at 5-min intervals, and steady state was reached after 95 to 120 min (Supplementary Fig. 1*A*). Whole-body insulin sensitivity (M-value) was assessed as the mean glucose infusion rate adjusted for glucose space correction (mg/kg fat-free mass/min) during the steady state (18). Mean plasma insulin levels were comparable during steady state (Supplementary Fig. 1*B*). The M-value/insulin (M/I) ratio was calculated as the M-value divided by the mean insulin concentrations during steady state for every participant.

Maximal Aerobic Capacity

$V_{O_{2peak}}$ was determined by an incremental treadmill test on a Trackmaster TMX 425c (Full Vision, Inc., Newton, KS) as previously described (19).

Body Composition and Blood Analyses

Body composition was measured by DXA using a Lunar iDXA Whole-Body Scanner (GE Healthcare Lunar, Madison, WI) (2). Fasting and steady-state (clamp) blood samples were analyzed in clinical chemistry laboratory at either Florida Hospital or onsite at the Translational Research Institute for Metabolism and Diabetes, where appropriate (20).

Muscle Biopsy

Muscle biopsies were performed in vastus lateralis muscle prior to training. Muscle tissue was either snap frozen or used for the isolation of myogenic progenitor cells (20,21).

Myogenic Progenitor Cell Isolation and Differentiation

Myogenic progenitor cells (HSkMCs) were isolated as described previously (22). HSkMCs (myoblasts) were immunopurified using mouse monoclonal 5.1H11 anti-CD56 antibody (23). Myoblasts were differentiated into myotubes for 5–7 days (22).

Genome-Wide DNA Methylation Analysis

Total DNA was analyzed in all nonresponders (n = 6) and in the responders with the most prominent increase in PCr recovery rate with training (n = 6). DNA was isolated from pretraining muscle tissue and myotubes using the DNeasy Blood and Tissue Extraction Kit (Qiagen, Valencia, CA). Genome-wide methylation analysis was performed using the Illumina Infinium MethylationEPIC BeadChip platform, which interrogates > 850,000 CpG methylation sites. Raw data were summarized into BeadStudio IDAT files for further analysis using the Partek Genomic Suite (Partek, Inc., St. Louis, MO). Data were normalized using the SWAN (Subset-Quantile With Array Normalization) method. Differentially methylated CpG sites between nonresponders and responders were identified by ANOVA using relaxed conditions to define differentially methylated CpG sites (unadjusted P value cutoff = 0.05; estimated change in β-value cutoff ≥0.2, corresponding to a ≥20% change in methylation between groups). Corresponding genes were used for enrichment analyses.

RNA Sequencing Analyses

Total RNA was analyzed in pretraining muscle tissue and myotubes of all nonresponders (n = 6) and in those of responders (n = 6; same participants as for DNA methylation) with the most prominent increase in PCr recovery rate with training, and isolated using RNeasy Fibrous Tissue kit (Qiagen). Raw data were quality controlled by FastQC. Mapping to human genome (UCSC hg19) was performed using the Illumina Cufflinks Assembly & DE workflow. Abundance estimates for genes defined in a genome reference, FPKM (fragments per kilobase of transcript per million mapped reads), were used for downstream analysis. Data were further filtered as generally recommended: low expressed genes were removed (genes with max FPKM value for all samples ≤1), and FPKM signals were further log2 transformed. Genes were removed based on a cutoff for SD of log2 of signals for all samples ≤0.2. Differentially expressed genes (DEGs) between nonresponders and responders were defined using ANOVA (unadjusted P value <0.05 and fold change >1.5).

Enrichment analyses were subsequently performed.

Statistical Analyses

Values are reported as the mean ± SD. Statistical significance was set at $P < 0.050$. An unpaired Student *t* test was used to analyze group differences pretraining. Repeated measures were analyzed using a two-way repeated-measures ANOVA model. When normality was violated, the appropriate nonparametric test was performed. An ANOVA was used to analyze pretraining differences in DNA methylation and RNA expression. A χ^2 test was used to analyze group distributions for sex, race, and medication use. For associations between variables, Pearson's correlations were performed. All statistical analyses were performed using JMP version 13 (SAS Institute, Cary, NC), Partek NSG (Partek, Inc.), and Prism 6 (GraphPad, San Diego, CA).

RESULTS

Participant Characteristics

Individuals with type 2 diabetes ($n = 17$) were classified as nonresponders ($n = 6$) or responders ($n = 11$) based on whether PCr recovery rate decreased or increased with training, respectively (Fig. 1*A*). Pretraining characteristics of nonresponders and responders are summarized in Table 1. Nonresponders and responders did not differ in age ($P = 0.245$), sex ($P = 0.858$), or race ($P = 0.493$). BMI was comparable between nonresponders and responders at the time of study enrollment (34.3 ± 4.2 and 35.2 ± 3.7 kg/m^2, $P = 0.676$, for nonresponders and responders, respectively). The duration of type 2 diabetes was similar between nonresponders and responders ($P = 0.737$). Nonresponders had higher PCr recovery rates ($P = 0.005$) (Fig. 1*B*) and higher insulin sensitivity ($P = 0.041$) (Fig. 1*D*) than responders pretraining. Values for HbA_{1c} ($P = 0.769$) (Fig. 1*F*), $V_{O_{2peak}}$ ($P = 0.571$) (Fig. 1*H*), fasting plasma glucose ($P = 0.575$), fasting insulin ($P = 0.201$), total cholesterol ($P = 0.909$), LDL cholesterol (LDL-C; $P = 0.767$), HDL cholesterol (HDL-C; $P = 0.618$), triglycerides ($P = 0.356$), fasting plasma free fatty acids ($P = 0.998$), body weight ($P = 0.512$), fat mass ($P = 0.725$), and fat-free mass ($P = 0.640$) were not different between nonresponders and responders pretraining. Pretraining PCr recovery rate correlated positively with pretraining insulin sensitivity ($r = 0.558$, $P = 0.020$) (Fig. 1*J*).

Compliance, Adherence, and Medication Use

Values for compliance with (percentage of training sessions completed of 40 in total) and adherence to (percentage of time spent in the individual target heart rate zone during each training session) the training protocol were similar between nonresponders and responders ($P = 0.571$ and $P = 0.958$, respectively) (Table 1). Caloric intake was not significantly different between nonresponders and responders pretraining ($P = 0.762$) (Table 1). Glucose-lowering medication use before the start of the study was similar between groups, with 67% of nonresponders and 64% of responders using metformin alone (Table 1).

Training-Induced Changes

By design, the PCr recovery rate in nonresponders decreased ($P = 0.031$), whereas the PCr recovery rate in responders increased ($P = 0.001$) after 10 weeks of aerobic training, and the training-induced relative change in PCr recovery rate was significantly different between nonresponders and responders ($P < 0.001$) (Fig. 1*C* and Table 1). Unpublished data from our laboratory demonstrate that there are no significant changes in PCr recovery rate measured 3–4 weeks apart without intervention (i.e., free living) in the same healthy sedentary individuals. Insulin sensitivity increased in responders ($P = 0.032$), but not in nonresponders ($P = 0.438$) (Fig. 1*E* and Table 1). Training-induced absolute changes in (Δ) insulin sensitivity positively correlated with ΔPCr recovery rate ($r = 0.517$, $P = 0.034$), and percentage changes in insulin sensitivity positively correlated with percentage changes in PCr recovery rate ($r = 0.498$, $P = 0.042$). HbA_{1c} increased in nonresponders ($P = 0.004$), but not in responders ($P = 0.999$), and the training-induced relative change in HbA_{1c} was significantly different between nonresponders and responders ($P = 0.002$) (Fig. 1*G* and Table 1). ΔHbA_{1c} negatively correlated with the ΔPCr recovery rate ($r = -0.690$, $P = 0.019$), and percentage changes in HbA_{1c} negatively correlated with percentage changes in PCr recovery rate ($r = -0.727$, $P = 0.011$). $V_{O_{2peak}}$ significantly increased in both nonresponders (+14.21%) and responders (10.37%) after 10 weeks of training ($P < 0.001$) (Fig. 1*I* and Table 1). Body weight ($P = 0.017$) and fat-free mass ($P = 0.005$) increased in nonresponders, and the relative changes were different between nonresponders and responders (Table 1). Values for fat mass ($P = 0.429$), plasma glucose ($P = 0.388$), plasma insulin ($P = 0.985$), total cholesterol ($P = 0.558$), LDL-C ($P = 0.979$), HDL-C ($P = 0.346$), triglycerides ($P = 0.110$), and plasma free fatty acids ($P = 0.578$) under fasting conditions did not significantly change with training in either group (Table 1). Caloric intake did not significantly change with training in either group ($P = 0.695$) (Table 1).

Pretraining Genome-Wide DNA Methylation and RNA Sequencing Analyses

We investigated the potential epigenomic and transcriptomic contributions to the training response variation in muscle mitochondrial function in vivo and insulin sensitivity. To this end, global DNA methylation of the inner cytosine within the CCGG sequence (CpG methylation) and RNA expression patterns were assessed in muscle tissue pretraining. Striking differences in DNA methylation patterns, representing 533 differentially methylated CpG sites, were observed between nonresponders and responders (Fig. 2*A* and Supplementary Table 1). Of those 533 differentially methylated CpG sites, methylation levels were significantly lower in the promoter region (TSS200 region, $P = 0.024$) (Fig. 2*B*) and in the CpG island region ($P = 0.004$) (Fig. 2*C*), which tends to overlap with the promoter regions (24), of nonresponders versus responders. RNA sequencing (RNAseq) analyses revealed a similar pattern (similar to DNA methylation) in nonresponders compared to that in responders, representing 118 DEGs (heatmap not shown) (Supplementary Table 3). Global DNA methylation levels in these regions in muscle tissue showed significant correlations with pretraining values for PCr recovery rate and insulin sensitivity (Supplementary Fig. 2) and with ΔPCr recovery rate and ΔHbA_{1c} (Supplementary Fig. 3). Enrichment analyses of both DNA methylation and RNAseq data identified pathways linked to glutathione metabolism, insulin signaling, and mitochondrial metabolism. The calculated

Table 1—Pretraining values and percentage changes from pretraining

	Pretraining		Change (%)	
	Nonresponders	Responders	Nonresponders	Responders
Age (years)	47.2 ± 9.5	52.6 ± 6.6		
Sex (F/M), *n*	3/3	5/6		
Sex (F/M), %	50/50	45/55		
Race (black/white/Hispanic/unknown), *n*	2/4/0/0	1/8/1/1		
Race (black/white/Hispanic/unknown), %	33/67/0/0	9/73/9/9		
Diabetes duration (years)	4.67 ± 3.20	5.45 ± 5.07		
Caloric intake (kcal/day)	1,912 ± 468	2,015 ± 578	−7.18 ± 27.27	14.33 ± 31.16
PCr recovery rate (1/s)	0.026 ± 0.005[A]	0.017 ± 0.004[B]	−23.92 ± 12.28*[A]	31.64 ± 37.23*[B]
M-value (mg/kg fat-free mass/min)	6.5 ± 2.9A	3.6 ± 2.3B	−13.52 ± 41.3	102.69 ± 211.41*
M/I	0.040 ± 0.016[A]	0.019 ± 0.012[B]	−14.04 ± 47.13	94.48 ± 172.01*
HbA_{1c} (%)	7.4 ± 0.9	7.6 ± 0.8	11.09 ± 5.49*[A]	−0.36 ± 7.83[B]
HbA_{1c} (mmol/mol)	57 ± 9.8	60 ± 8.7	11.09 ± 5.49*[A]	−0.36 ± 7.83[B]
Vo_{2peak} (mL/kg/min)	20.3 ± 4.8	22.0 ± 4.6	14.21 ± 5.24*	10.37 ± 7.77*
Fasting plasma glucose (mmol/L)	9.7 ± 2.3	10.6 ± 2.9	13.81 ± 14.73	−2.49 ± 24.93
Fasting insulin (μIU/mL)	16.7 ± 5.5	13.1 ± 3.6	−6.51 ± 56.89	6.04 ± 36.17
Total cholesterol (mmol/L)	4.0 ± 0.9	4.0 ± 0.5	4.40 ± 18.77	3.15 ± 11.32
LDL-C (mmol/L)	2.3 ± 0.6	2.4 ± 0.5	3.80 ± 29.14	−3.45 ± 16.03
HDL-C (mmol/L)	0.9 ± 0.3	0.9 ± 0.1	6.67 ± 8.93	−0.43 ± 11.85
Triglycerides (mmol/L)	1.7 ± 0.4	1.4 ± 0.5	10.55 ± 31.79	28.32 ± 41.36
Fasting plasma free fatty acids (mmol/L)	0.50 ± 0.27	0.50 ± 0.14	2.63 ± 22.36	5.99 ± 10.96
Body weight (kg)	96.8 ± 13.6	101.7 ± 15.4	2.57 ± 2.65*[A]	−0.08 ± 1.68[B]
Fat mass (kg)	39.5 ± 9.3	41.2 ± 8.7	2.77 ± 4.49	−1.13 ± 3.69
Fat-free mass (kg)	57.3 ± 14.5	60.5 ± 10.1	2.87 ± 2.43*[A]	0.56 ± 1.48[B]
Compliance (%)	92.6 ± 4.8	94.4 ± 6.6		
Adherence[a] (%)	76.8 ± 11.2	77.2 ± 12.8		
Type 2 diabetes medications, *n* (%)				
Metformin	4 (67)	7 (64)		
Metformin + sulfonylurea	2 (33)	3 (27)		
Diet	0 (0)	1 (9)		

Data are the mean ± SD, unless otherwise indicated. F, female; M, male. [a]Recorded every 5 min. *$P < 0.050$ pretraining vs. post-training within the same group; different capital letters [A and B] indicate significant differences between nonresponders and responders ($P < 0.050$).

P values for the 533 differentially methylated CpG sites and their chromosomal locations are shown in Fig. 2*D*. The glutathione S-transferase μ (GSTM) class gene family dominated both DNA methylation and RNA expression patterns. Twelve CpG sites for GSTM1 and GSTM5 were located within the gene promotor regions (chromosome 1) (Fig. 2*D*). The inset in Fig. 2*D* shows that methylation in all 12 CpG sites located on chromosome 1 for GSTM1 and GSTM5 were downregulated in nonresponders compared with responders (fold change). Methylation levels in nonresponders and responders for those 12 CpG sites are shown quantitatively in Fig. 2*E*. Corresponding RNA expressions for GSTM1, GSTM4, and GSTO1 were significantly higher in nonresponders and responders (Fig. 2*F*). In the insulin signaling pathway, we observed differentially methylated CpG sites in the promotor regions of microfibril-associated protein 3 like (MFAP3 L), phosphofructokinase muscle, and phosphoinositide-3-kinase regulatory subunit 3 (Fig. 2*E*). RNA expression for MFAP3 L was significantly lower in nonresponders compared with responders (Fig. 2*F*). RNA expressions for numerous subunits of the electron transport system complexes were differentially expressed between nonresponders and responders, being significantly higher in nonresponders (Fig. 2*F*), as follows: NADH-ubiquinone oxidoreductase subunit A2 (NDUFA2), NDUFA7, NDUFA9, NDUFA11, and ubiquinol-cytochrome c reductase hinge protein (UQCRHL). Pyruvate dehydrogenase kinase 4 (PDK4) and growth arrest and DNA damage inducible α (GADD45A), genes related to mitochondrial metabolism, were differentially expressed in nonresponders versus responders (Fig. 2*F*). In general, DNA methylation in the promoter regions of genes corresponded with a reciprocal RNA expression pattern. The DNA methylation and RNA expression patterns in pretraining muscle tissue suggest higher antioxidant defense, insulin signaling, and mitochondrial metabolism in nonresponders prior to the training intervention, which is reflected in vivo by means of higher pretraining PCr recovery rate and higher pretraining insulin sensitivity in nonresponders compared with responders.

To determine whether these epigenomic patterns were intrinsic to the muscle, we assessed global DNA methylation and RNA expressions in myogenic progenitor cells (HSkMCs) derived from these same individuals pretraining. A total of 491 CpG sites were differentially methylated in HSkMCs from nonresponder compared with responders,

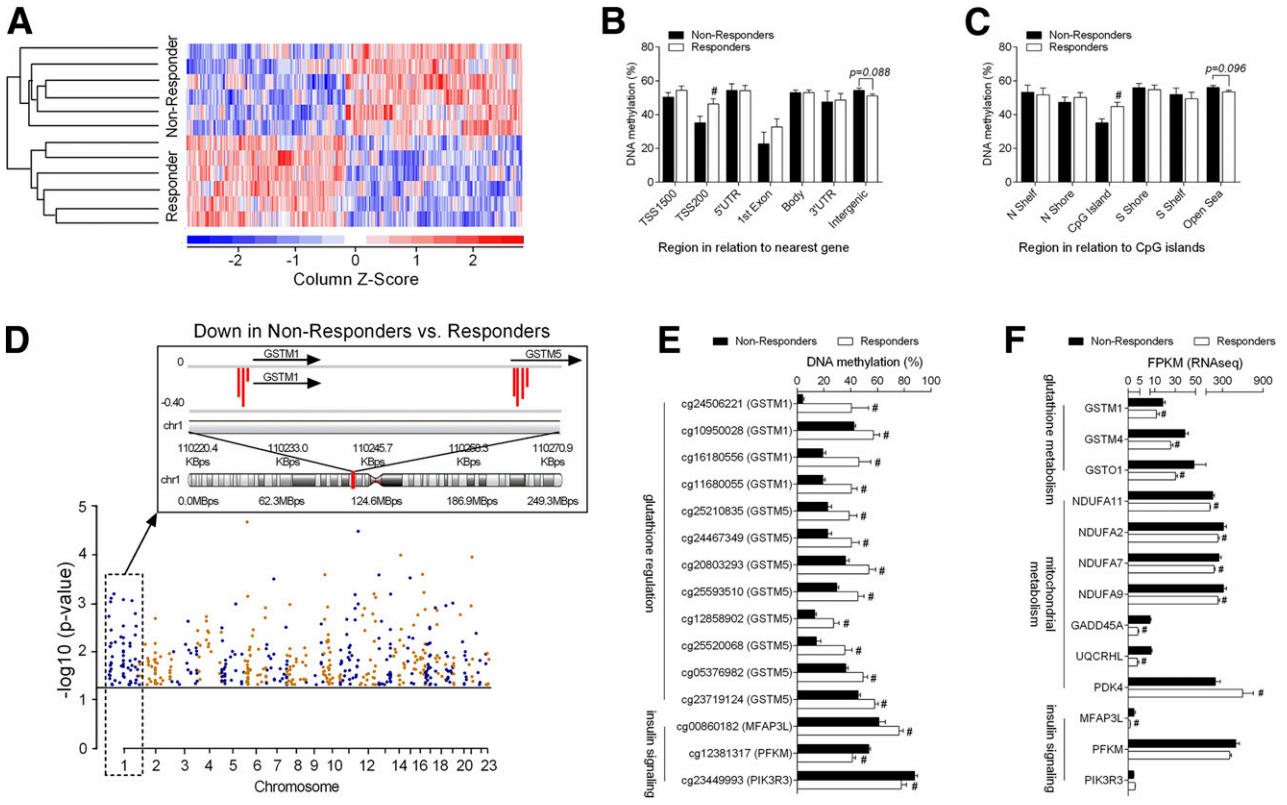

Figure 2—Pretraining DNA methylation and gene expression in muscle tissue. In total, 533 individual CpG sites were differentially methylated in pretraining muscle tissue of nonresponders vs. responders as depicted in the heatmap (*A*). *B*: Calculating the average methylation level for these 533 differentially methylated CpG sites in muscle tissue based on the functional genome revealed lower DNA methylation in the promotor region for these genes in nonresponders vs. responders. *C*: Calculating the average methylation level for these 533 differentially methylated CpG sites in muscle tissue based on the CpG content and neighborhood content revealed lower DNA methylation in the CpG island region, which is known to overlap with the promotor regions. *D*: Manhattan plot of the *P* values calculated from the genome-wide CpG site methylation analysis in muscle tissue on all chromosomes (n = 533 differentially methylated CpG sites in total), and the inset shows the fold changes of the 12 CpG sites differentially methylated in GSTM1 and GSTM5 on chromosome 1 for nonresponders vs. responders. *E*: CpG sites in the promotor region of genes related to glutathione metabolism and insulin signaling were differentially methylated in muscle tissue of nonresponders vs. responders, with lower DNA methylation in the promotor region of these genes in nonresponders vs. responders. *F*: Transcript levels (FPKM values from RNAseq data) for genes involved in glutathione metabolism, mitochondrial metabolism, and insulin signaling were different in muscle tissue of nonresponders vs. responders, with higher expressions in nonresponders vs. responders. Data are mean ± SD, where appropriate. #$P < 0.050$ compared with nonresponders.

with 113 CpG sites in common between pretraining muscle tissue and HSkMCs derived from the same tissue of the same individuals (Fig. 3*A* and Supplementary Table 2). In accordance with muscle tissue, of the 491 differentially methylated CpG sites, methylation levels in HSkMCs were lower in the promoter region (TSS1500 region, $P = 0.005$; TSS200 region, $P = 0.062$) (Fig. 3*B*) and the CpG island region ($P < 0.001$) (Fig. 3*C*) of nonresponders compared with responders. Global DNA methylation levels in these regions in HSkMCs showed significant correlations with pretraining and ΔPCr recovery rate and HbA_{1c} (Supplementary Figs. 4 and 5). Enrichment analysis identified pathways related to glutathione metabolism. The calculated *P* values for the 491 differentially methylated CpG sites and their chromosomal locations are shown in Fig. 3*D*. Three CpG sites in GSTM1 and three CpG sites in GSTM5 were located within the promotor regions (chromosome 1) (Fig. 3*D*) and downregulated in nonresponders compared with responders (fold change; inset in Fig. 3*D*). Methylation levels in nonresponders and responders for those six CpG sites are shown quantitatively in Fig. 3*E*. DNA methylation in the promotor regions of protein kinase C α, phospholipase C β 4 (PLCB4) and PLCL2 related to insulin signaling and for NDUFA12 related to mitochondrial metabolism were also different in nonresponders compared with responders (Fig. 3*E*). RNAseq analyses in the pretraining HSkMCs (n = 147) (Supplementary Table 4) showed an overlap of five DEGs in nonresponders compared with responders with the pretraining muscle tissue DEGs (n = 118) (Supplementary Table 3). Of the 147 total DEGs in pretraining HSkMCs, one gene was also differentially methylated in the HSkMCs (PLCL2). RNA expressions of selected genes relevant to glutathione regulation, mitochondrial metabolism, and insulin signaling are shown quantitatively in Fig. 3*F*. Interestingly, GSTM1 is higher ($P = 0.070$) in pretraining HSkMCs from nonresponders compared with responders (Fig. 3*F*), which corresponds to the findings in muscle tissue GSTM1 RNA expression (Fig. 2*F*), as well as reduced DNA methylation in the promoter region of GSTM1 in both pretraining muscle tissue and HSkMCs (Figs. 2*E* and 3*E*). Thus, the training response variation observed in the epigenomic profiles of muscle tissue appeared to be cell autonomous.

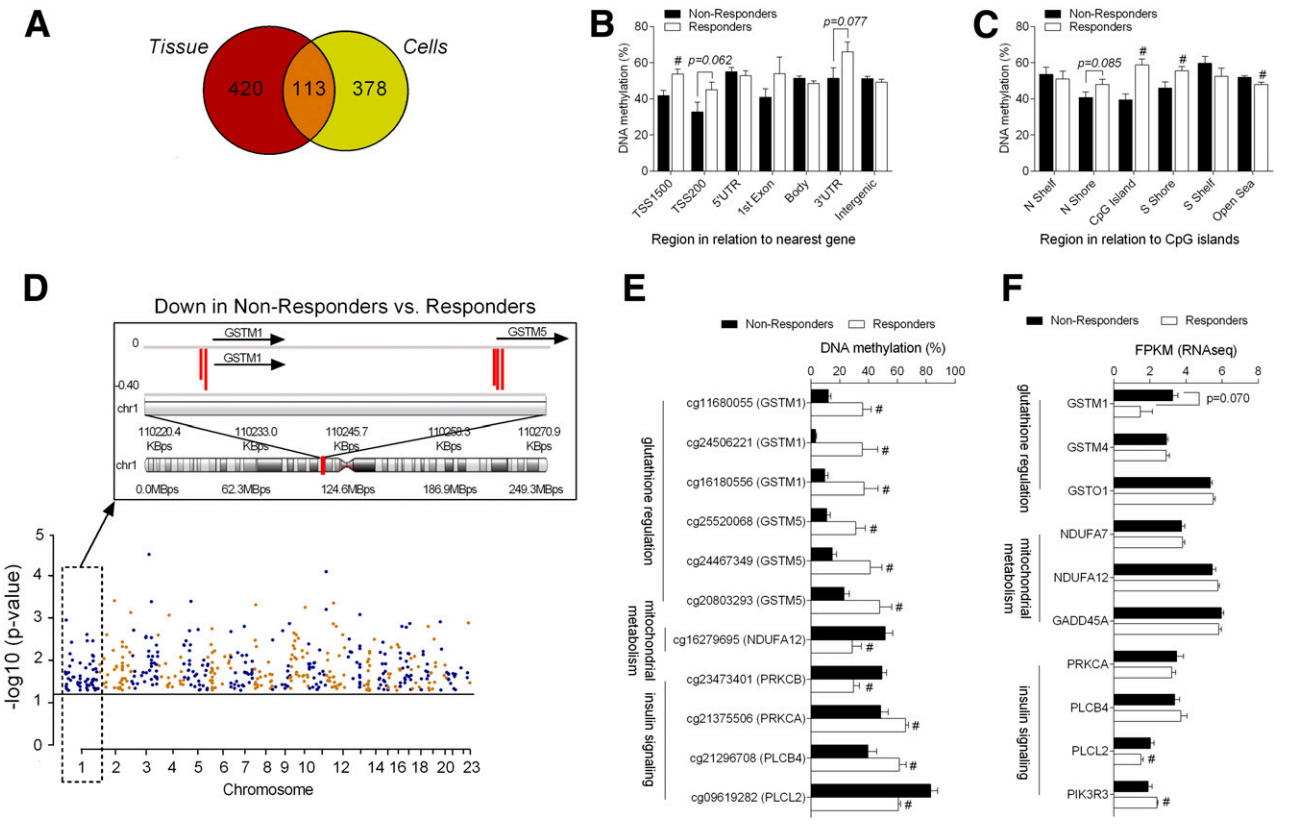

Figure 3—Differences in pretraining DNA methylation were preserved in myogenic progenitor cells. Some differences in the methylation of CpG sites in muscle tissue were preserved in myogenic progenitor cells (HSkMCs). In total, 420 CpG sites were differentially methylated only in muscle tissue (red), 378 CpG sites were differentially methylated only in HSkMCs (yellow), and 113 differentially methylated CpG sites were in common between muscle tissue and HSkMCs (orange) of nonresponders vs. responders (*A*). *B*: Calculating the average methylation level for these 491 differentially methylated CpG sites in HSkMCs based on the functional genome revealed lower methylation in the promotor region for these genes in nonresponders vs. responders. *C*: Calculating the average methylation level for these 491 differentially methylated CpG sites in HSkMCs based on the CpG content and neighborhood content revealed lower methylation in the CpG island region, which is known to overlap with the promotor regions. *D*: Manhattan plot of the *P* values calculated from the genome-wide CpG site methylation analysis in HSkMCs (n = 491 sites across all chromosomes). *E*: As in muscle tissue, CpG sites in the promotor regions of genes related to glutathione metabolism, mitochondrial metabolism, and insulin signaling were differentially methylated in HSkMCs in nonresponders vs. responders, with lower methylation in the promotor region of these genes in nonresponders vs. responders. *F*: Transcript levels (FPKM values from RNAseq data) for genes involved in glutathione metabolism, mitochondrial metabolism, and insulin signaling in HSkMCs of nonresponders vs. responders. Data are mean ± SD, where appropriate. #$P < 0.050$ compared with nonresponders.

CONCLUSIONS

We used training response variation in muscle mitochondrial function in vivo (measured by PCr recovery rate) after 10 weeks of aerobic training to classify individuals with type 2 diabetes as nonresponders or responders. Individuals classified as nonresponders did not improve insulin sensitivity and worsened glycemic control with training, whereas responders improved insulin sensitivity. The training response variation in muscle mitochondrial function in vivo was marked by a distinct pretraining molecular pattern in muscle tissue and in myogenic progenitor cells (HSkMCs) of nonresponders compared with responders. DNA methylation and RNA expression patterns showed elevations in antioxidant defense, insulin signaling, and mitochondrial metabolism in nonresponders, which were reflected in vivo by higher pretraining muscle mitochondrial function and insulin sensitivity in these same individuals.

On average, exercise-training interventions improve muscle mitochondrial function (25,26) and even restore muscle mitochondrial function in individuals with type 2 diabetes to levels observed in healthy individuals (4,27). Conclusions about the beneficial effects of exercise training are too often drawn based on average responses, and little is known about those individuals who do not respond favorably to exercise training. Here we identified individuals with type 2 diabetes who did not improve muscle mitochondrial function in vivo with training that correlated with a lack of improvement in insulin sensitivity and worsened glycemic control. In a previous study, first-degree relatives of individuals with type 2 diabetes who did not improve muscle ATP synthesis rates in vivo after three bouts of exercise did not improve insulin sensitivity, whereas those who improved in vivo muscle ATP synthesis rates also improved insulin sensitivity (8). Evidence for a cause-and-effect relationship between muscle mitochondrial function and insulin resistance is still limited (25). However, it is well documented that, on average, improvements in muscle mitochondrial function induced by exercise training in individuals with type 2 diabetes are paralleled by improvements in insulin sensitivity (4,25–27). Our data expand these previous findings by demonstrating that the

variation in training response in muscle mitochondrial function in vivo also corresponds to the variation in training response in insulin sensitivity in individuals with type 2 diabetes.

Interest in the topic of biological individuality has a long history (28). Although the emphasis has been on nutrition and nutrients, its basic concept is relevant for adaptations to exercise training as well. The concept of training response variation was proposed 35 years ago (29) and has been traditionally defined as the interindividual variation in changes in $V\text{O}_{2max}$ with exercise training. The most comprehensive data on training response variation related to $V\text{O}_{2max}$ comes from the HERITAGE Family Study (30). In this study, exercise training increased $V\text{O}_{2max}$ for the groups, but individual changes were highly variable, such that some individuals did not improve $V\text{O}_{2max}$ with training. Low $V\text{O}_{2max}$ is associated with metabolic risk factors present in type 2 diabetes, such as aberrant muscle mitochondrial function, insulin resistance, and hyperglycemia (2,31). The relationship between training-induced changes in $V\text{O}_{2max}$ and training-induced changes in metabolic risk factors, however, is not completely clear. Some studies showed associations between training-induced changes in $V\text{O}_{2max}$ and metabolic risk factors (32), whereas others failed to show this association (33,34). In the current study, we found that nonresponders had similar improvements in $V\text{O}_{2peak}$ compared with responders. In a previous exercise training study (33), only one in every three individuals with type 2 diabetes showed improved $V\text{O}_{2max}$, but all showed decreased HbA_{1c} levels. Thus, it seems that an association between training responses in $V\text{O}_{2max}$ and metabolic risk factors is not apparent for every individual. Moreover, our data suggest that training response variation related to metabolic risk factors seems to be independent of the training response variation related to cardiorespiratory fitness. Identifying the training response variation related to metabolic risk factors in individuals with type 2 diabetes could inform future combinations of lifestyle and pharmaceutical therapeutic strategies aimed at resolving metabolic complications of type 2 diabetes (35).

Nonresponders were characterized with better in vivo muscle mitochondrial function and higher insulin sensitivity pretraining than responders. There is some previous evidence suggesting that individuals with a favorable metabolic profile benefit less from exercise or lifestyle interventions than individuals with a higher metabolic burden (32). For example, first-degree relatives of individuals with type 2 diabetes who did not improve in vivo muscle ATP synthesis rates after three bouts of exercise were characterized with higher muscle ATP synthesis rates at baseline compared with individuals who improved muscle ATP synthesis rates (8). On the other hand, unfavorable glucose homeostasis before training blunted training-induced improvements in 2-h post–oral glucose tolerance test blood glucose levels (36) and insulin sensitivity (37), suggesting that individuals with an unfavorable metabolic profile at baseline benefit less from exercise interventions. These conflicting results might be explained by a ceiling effect or a regression to the mean for some variables, different populations, and different study designs. These findings, together with the data presented herein, are just the tip of the iceberg, and more research is warranted in this exciting area of investigation to better understand the impact of preintervention metabolic profiles on the exercise response in hopes of developing biomarkers as potential predictors of the exercise response. High plasma free fatty acid levels have also been linked to impaired muscle mitochondrial function and insulin resistance (38,39); however, fasting plasma free fatty acid levels were not different between groups, nor did training influence plasma free fatty acid levels, suggesting no direct relationship between training response variability for muscle mitochondrial function in vivo and plasma free fatty acids levels.

Individuals within a family respond more similarly to exercise training in terms of metabolic risk factors than those between families, suggesting that hereditary factors contribute to the training response variability (6,11,12). A single muscle contraction induces hypomethylation in the promotor region—followed by transcriptional upregulation—of genes involved in mitochondrial metabolism, highlighting the potential epigenomic control of exercise training responses (14). We previously described distinct baseline (pretraining) transcriptional profiles in muscle tissue between individuals who did (responders) or did not (nonresponders) have improved HbA_{1c} levels, muscle mitochondrial content, body fat, and BMI after 9 months of supervised exercise training (10). In the current study, we identified distinct differences in the pretraining muscle tissue DNA methylation and transcriptional profiles of nonresponders versus responders, and differences in DNA methylation were specifically located within the promotor regions. Differences in DNA methylation in muscle tissue of nonresponders compared with responders largely overlapped with differences in DNA methylation in myogenic progenitor cells (HSkMCs) of these same individuals, demonstrating that these epigenomic differences are intrinsic to muscle. Our study is the first to investigate DNA methylation profiles in muscle tissue and myogenic progenitor cells in nonresponders and responders to exercise training and suggests that the training response variations observed in the epigenomic profiles of muscle tissue are cell autonomous.

The most robust differentially methylated and expressed gene set was linked to glutathione metabolism, with lower methylation and higher mRNA expressions of these genes in nonresponders compared with responders. Differences in glutathione metabolism in muscle tissue have been described in high and low responders to a diet intervention (40). Glutathione metabolism is essential for several antioxidant defense systems (41), and thus higher expressions of glutathione-metabolism-related genes suggests higher antioxidant activity in muscle. Training-induced oxidative stress responses seem to be important for the activation of signaling pathways related to the beneficial effects of exercise (42). Therefore, higher baseline antioxidant activity in the muscle of nonresponders might have contributed to the impaired training response in muscle mitochondrial function and insulin sensitivity. Enrichment analyses also revealed pathways linked to mitochondrial metabolism and insulin signaling in muscle tissue of nonresponders compared with responders prior to exercise training. Recent findings have shown that mitochondria are regulated by and can in turn regulate epigenomic mechanisms via mitonuclear communication (43). Changes in mitochondrial function, for example,

influenced the activity of *jmjd-1.2*/PHF8 and *jmjd-3.1*/JMJD3, two histone lysine demethylases that are part of an epigenomic mechanism that regulates life span (44). Our data suggest a connection between mitochondrial function and epigenomic modifications in the context of exercise training in humans. Upregulation of epigenomic signals involving mitochondrial metabolism and insulin signaling in nonresponders (vs. responders) at baseline aligned with higher mitochondrial function in vivo and insulin sensitivity in nonresponders (vs. responders) at baseline. Baseline epigenomic mechanisms in muscle may therefore serve as a key regulator of pathways that can influence an individual's metabolic exercise-training response.

In conclusion, training response variation in muscle mitochondrial function in vivo corresponds to training response variation for insulin sensitivity and glycemic control, but is independent of training response variation for cardiorespiratory fitness. A distinct pretraining molecular pattern in muscle tissue characterizes the training response variation in mitochondrial function in vivo, which includes differential enrichment of pathways linked to antioxidant defense, insulin signaling, and mitochondrial metabolism. These differences in pretraining epigenomic profiles were maintained in myogenic progenitor cells, suggesting that the training response variation observed in the epigenomic profiles of muscle tissue was cell autonomous. Our data provide new evidence to potentially shift the diabetes treatment paradigm by identifying individuals that do not reap metabolic benefits from exercise training, such that supplemental treatment options can be designed.

Acknowledgments. The authors thank the volunteers from the study for their participation. The authors also thank the recruitment team and all clinical personnel and all laboratory personnel at the Translational Research Institute for Metabolism and Diabetes.

Funding. This work is supported by a grant from the American Diabetes Association (#7-13-JF-53).

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. N.A.S. performed experiments and data analyses and critically reviewed and edited the manuscript. B.B. performed experiments and data analyses and wrote the manuscript. A.M.E. and F.Y. performed data analyses and critically reviewed and edited the manuscript. H.H.C. performed experiments and critically reviewed and edited the manuscript. C.M., B.H.G., and R.E.P. designed the study, analyzed the data, and critically reviewed and edited the manuscript. S.R.S. aided in study design and critically reviewed and edited the manuscript. L.M.S. designed the study, performed experiments, analyzed the data, and critically reviewed and edited the manuscript. L.M.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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