A Single Nucleotide Polymorphism Associates With the Response of Muscle ATP Synthesis to Long-Term Exercise Training in Relatives of Type 2 Diabetic Humans

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OBJECTIVE—Myocellular ATP synthesis (fATP) associates with insulin sensitivity in first-degree relatives of subjects with type 2 diabetes. Short-term endurance training can modify their fATP and insulin sensitivity. This study examines the effects of moderate long-term exercise using endurance or resistance training in this cohort.

RESEARCH DESIGN AND METHODS—A randomized, parallel-group trial tested 16 glucose-tolerant nonobese relatives (8 subjects in the endurance training group and 8 subjects in the resistance training group) before and after 26 weeks of endurance or resistance training. Exercise performance was assessed from power output and oxygen uptake (V̇O₂) during incremental tests and from maximal torque of knee flexors (MaxT flex) and extensors (MaxT ext) using isokinetic dynamometry. fATP and ectopic lipids were measured with 31P magnetic resonance spectroscopy.

RESULTS—Endurance training increased power output and V̇O₂ by 44 and 30%, respectively (both \( P < 0.001 \)), whereas resistance training increased MaxT flex and MaxT ext by 23 and 40%, respectively (both \( P < 0.001 \)). Across all groups, insulin sensitivity (382 ± 90 vs. 389 ± 40 \( \text{mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2} \)) and ectopic lipid contents were comparable after exercise training. However, 8 of 16 relatives had 26% greater fATP, increasing from 9.5 ± 2.3 to 11.9 ± 2.4 \( \text{μmol} \cdot \text{mL}^{-1} \cdot \text{m}^{-1} \) (\( P < 0.05 \)). Six of eight responders were carriers of the G/G single nucleotide polymorphism rs540467 of the NDUFB6 gene (\( P = 0.019 \)), which encodes a subunit of mitochondrial complex I.

CONCLUSIONS—Moderate exercise training for 6 months does not necessarily improve insulin sensitivity but may increase ATP synthase flux. Genetic predisposition can modify the individual response of the ATP synthase flux independent of insulin sensitivity.

First-degree relatives of patients with type 2 diabetes are at greater risk of developing type 2 diabetes, which has been mainly attributed to impaired insulin sensitivity. Insulin-resistant first-degree relatives have reduced insulin-mediated myocellular glucose-6-phosphate (G6P) and glycogen synthesis (1), and also may exhibit lower unidirectional flux through myocellular ATP synthase (fATP) (2), but elevated muscle and liver fat content (2). Lower insulin-stimulated fATP has been observed in overt type 2 diabetes (3,4), suggesting that altered ATP production could be involved in the progression of insulin resistance to diabetes.

In lean, insulin-resistant first-degree relatives, intensive endurance exercise training reduced insulin resistance by increasing insulin-stimulated G6P and glycogen synthesis (1). Such improvement of insulin resistance also has been associated with greater mitochondrial density and/or function in nondiabetic and type 2 diabetic individuals (5). However, the response of insulin sensitivity and mitochondrial function to exercise training may be dissociated depending on age (6) and endurance function (7). In addition, only one group of first-degree relatives (responders) can improve their insulin sensitivity and fATP after 1 week of endurance exercise training (8). The responder status was linked to the G/G single nucleotide polymorphism rs540467 in the NDUFB6 gene, which encodes a subunit of complex I of the respiratory chain, relates to insulin resistance and type 2 diabetes (9), and is less expressed in the skeletal muscle of type 2 diabetic patients (10). At present, it is unclear whether long-term endurance training can overcome this gene effect in nondiabetic first-degree relatives. Although intensive resistance exercise training also may ameliorate insulin resistance by increasing glucose transport and storage in type 2 diabetes (11), this also has not been reported in first-degree relatives. In addition to the type of exercise, frequency also affects...
the metabolic outcome of training programs. Lower training frequencies (i.e., twice weekly) may improve insulin resistance using either resistance training in older men with type 2 diabetes (12) or endurance training in obese women (13). Because training regimens may provide higher exercise adherence and practicability for sedentary people, we tested the hypotheses that long-term but moderate exercise training (1) improves fATP and insulin sensitivity, 2) overcomes the impact of the NDUFβ6 gene polymorphism observed during short-term exercise, and 3) that endurance training is superior to resistance training in increasing fATP in glucose-tolerant, nonobese first-degree relatives.

RESEARCH DESIGN AND METHODS—Twenty-four (50% female) eligible, glucose-tolerant, nonobese subjects with at least one parent with type 2 diabetes, confirmed by hyperglycemia or glucose-lowering treatment, were recruited from a group of first-degree relatives who had participated in a previous 1-week study (8). By design, the 26-week exercise training immediately followed the 1-week exercise training. Four of these first-degree relatives declined to participate in the training study, and another four subjects (two from the endurance training group and two from the resistance training group) did not finish the study because of missing motivation (n = 2), depression (n = 1), and viral infection (n = 1) (Supplementary Fig 1). Consequently, 16 (50% female) subjects with one (n = 12) or two (n = 4) parents with type 2 diabetes completed the study.

Subjects underwent medical history and physical examinations, including measurement of body weight, height, waist-to-hip ratio (WHR), and 12-lead electrocardiogram; blood and urine analyses; and oral glucose tolerance testing. Exclusion and inclusion criteria have been described (8). Women were neither taking hormonal contraceptives nor were they studied in the luteal phase of their cycle. Inclusion required written informed consent by the volunteer to the study, which was approved by the local institutional ethical board and performed according to the Declaration of Helsinki. Then, participants were randomly assigned to either endurance or resistance training interventions.

Dietary assessment
Dietary intake was assessed at baseline and during weeks 13 and 27 with a modified interviewer-administered, 107-item, open-ended food frequency questionnaire adjusted for local dietary habits (8). Nutrient and fluid intake before the studies were assessed from 24-h recalls. All volunteers were advised to follow a weight-maintaining diet as recommended by the American Diabetes Association (14).

Oral glucose tolerance test
Fasting insulin sensitivity was assessed with the quantitative insulin sensitivity check index, obtained as the inverse of the sum of the logarithms of fasting plasma insulin and glucose (13). A 75-g oral glucose tolerance test (OGTT) was performed for 150 min (8) to assess the dynamic insulin sensitivity with the oral glucose insulin sensitivity index (OGIS). OGIS, obtained from a model-derived equation (16) (http://www.isib.cnrm/bioing/ogis/home.html), describes glucose clearance and has been validated against the clamp and other insulin sensitivity indices (16,17). OGTT-derived parameters already have been used in our previous exercise study (8,16). β-Cell function was assessed with the insulinogetic index and with the adaptation index, the product of OGIS and insulin secretion from C-peptide (15). The latter describes how the β-cell adapts its glucose-stimulated response to changes of insulin resistance. Hepatic insulin extraction was calculated as previously reported (18).

Magnetic resonance spectroscopy
Participants were studied using a 3-Tesla magnetic resonance spectrometer (Medspec S 300-DBX; Bruker Biospin, Ettlingen, Germany). The procedures of 1H and 31P magnetic resonance spectroscopy (MRS) have been described previously (8). Data for one participant of the resistance training group could not be obtained at the end of the study as a result of a technical failure of the spectrometer.

Habitual physical activity, strength, and exercise testing
Physical activity was assessed with an interviewer-administered questionnaire on a scale from one to five (low to high degree of activity) (8).

Strength testing (isokinetic torque measures).
Maximal isokinetic muscle strength (peak torque) of extension and flexion at the knee was measured via isokinetic dynamometry (Lido Active Multijoint II; Loredan Biomedical, Sacramento, CA) in both legs. In brief, after warm-up and familiarization, the volunteers were instructed to maximally push and pull through the full available range of motion (20° to 90°). Every test included two reciprocal bouts with a 10-s rest period after each bout and a 2-min resting period between the preset test velocities (30°/s and 60°/s). The highest absolute peak torques obtained at each velocity from both limbs were recorded for extension and flexion movements.

Exercise testing (incremental tests).
This test was performed using an electronically braked cycle ergometer (Excalibur Sport; Lode, Groningen, Netherlands) at 70 revolutions per min in an upright position to the limit of tolerance. Respiratory gas exchange was assessed with an open-air spirometry system (MasterScreen CPX; Jager/Vasys, Wurzburg, Germany) as described (8). From incremental tests power output, oxygen uptake (V̇O2) was determined at maximum load and at the aerobic threshold (respiratory compensation point; RCP) (19).

Experimental protocols
All participants were advised to refrain from any physical exercise and to ingest carbohydrate-enriched meals during the 3 days before the start of all tests. In the evenings prior to the tests, the participants consumed identically composed carbohydrate-enriched dinners at identical times and then fasted for 12 h. On day 1, participants did not consume any calories except for the glucose provided during the OGTT until the end of the MRS. They underwent baseline blood sampling, OGTTs, and MRS. On day 2, they performed isokinetic strength tests, and 2 h later they performed the incremental exercise tests. On days 3 and 5, they exercised on a cycling ergometer. On day 7, measurements of day 1 were repeated in identical fashion as described (8). Thereafter, participants were randomly assigned to individually controlled endurance or resistance training, which started 1 week later, with every session supervised and documented by a coach. Blood checks and OGTTs were repeated in week 13. At the end of the study, after 26 weeks of training and 48 h after the last exercise, all measurements from day 1 were repeated in identical fashion (Supplementary Fig 2). The experimental protocol was designed to yield significant increases in exercise capacities for both endurance and resistance training (Supplementary Fig 3), as previously reported in humans with obesity or type 2 diabetes (12,13).

Endurance training group. Volunteers performed two sessions weekly, with increasing duration from 15 min up to 60 min per session. After the 1st week, the intensity of 80% was raised to 90% of the workload determined at the RCP.
Resistance training group. Participants lifted the greatest possible load for a pre-determined number of 12–15 correct repetitions (12–15 RM test) of eight different exercises involving all major lower- and upper-body and trunk muscles on exercise machines (Technogym; Gambettola, Forlì, Italy) to predict 1-repetition maximum (1RM) with reassessment of 12–15 RM tests every 4 weeks. During weeks 1–4, the intensity was increased from 30 to 50% 1RM. During weeks 5–18, the number of repetitions was raised to 15 for each of the eight exercises. From week 15 on, participants performed two sets of exercises. Starting with week 19, the intensity was increased to 70% 1RM (two sets of 15 repetitions until week 22, increasing to three sets until the end of the training intervention).

Laboratory analyses
Plasma glucose (Glucose analyzer II; Beckman Coulter, http://www.beckmancoulter.com), insulin, and C-peptide and all other parameters were analyzed as described (8). Serum total adiponectin concentrations were measured using the Quantikine Total Human Adiponectin ELISA Kit (R&D Systems, Wiesbaden, Germany) in a subgroup of study participants (five subjects from the resistance training group and six subjects from the endurance training group) for whom sufficient serum was available.

Genotyping
DNA was extracted from blood with a QIAamp DNA Blood Mini Kit (cat. no. 51106; Qiagen, Hilding, Germany); rs540467 of NDUF6 was genotyped using an allelic discrimination assay performed with an ABI 7900 system (Applied Biosystems, Foster City, CA) and an assay on demand (C_2334430; Applied Biosystems) (9).

Statistics
Statistical analyses were performed using Statistica software (version 6.1; StatSoft, Tulsa, OK). Data were expressed as means ± SD. Unpaired two-tailed Student t tests were used for between-group comparisons, paired t tests were used for comparing pretraining (baseline) with posttraining data within each training group. The association between rs540467 and exercise responses of fATP was analyzed with the χ2 test (NCSS Statistical Software, Kaysville, UT). P values ≤0.05 were considered to indicate significant differences between groups.

RESULTS
Baseline analyses
Both the endurance and resistance training groups did not differ in anthropometric, metabolic, and lifestyle parameters (Table 1). During the OGTT, glucose and insulin concentrations also were similar (Fig. 1). None of the participants fulfilled the current criteria for metabolic syndrome. No significant differences comparing men and women were found. Serum adiponectin was not different between endurance and resistance training (12.74 ± 7.99 vs. 13.40 ± 9.29 μg/mL). Performance during strength and exercise testing, as well as fATP, intramyocellular lipids (IMCL), and hepatocellular lipids (HCL), also were not different between endurance and resistance training groups (Table 2).

Table 1—Anthropometric and laboratory data, parameters of insulin sensitivity and insulin secretion, and dietary intake before (baseline) and after 26 weeks of training

<table>
<thead>
<tr>
<th>Variables</th>
<th>Endurance training</th>
<th>Resistance training</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>After training</td>
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<tr>
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<tr>
<td>Age (years)</td>
<td>42.5 ± 11.0</td>
<td>+0.5</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>25.8 ± 2.1</td>
<td>25.8 ± 2.2</td>
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<tr>
<td>WHR</td>
<td>0.85 ± 0.05</td>
<td>0.83 ± 0.07</td>
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<tr>
<td>Fasting glucose (mg/dL)</td>
<td>91.2 ± 6.0</td>
<td>92.3 ± 4.6</td>
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<tr>
<td>2-h Glucose (mg/dL)</td>
<td>108 ± 26</td>
<td>109 ± 32</td>
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<tr>
<td>HbA1c (%)</td>
<td>5.53 ± 0.39</td>
<td>5.47 ± 0.34</td>
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<tr>
<td>Fasting insulin (μU/mL)</td>
<td>6.16 ± 3.46</td>
<td>6.68 ± 3.86</td>
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<tr>
<td>TSH (μU/mL)</td>
<td>1.59 ± 0.74</td>
<td>1.78 ± 0.94</td>
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<tr>
<td>OGIS (mL·min⁻¹·m⁻²)</td>
<td>386 ± 71</td>
<td>382 ± 90</td>
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<tr>
<td>QUICKI</td>
<td>0.47 ± 0.06</td>
<td>0.46 ± 0.08</td>
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<tr>
<td>Insulinogenic index</td>
<td>5.9 ± 2.5</td>
<td>3.8 ± 1.3</td>
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<tr>
<td>Adaptation index</td>
<td>7.92 ± 9.73</td>
<td>3.97 ± 1.90</td>
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<tr>
<td>Hepatic insulin extraction (%)</td>
<td>68.5 ± 10.9</td>
<td>63.1 ± 14.2</td>
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<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.16 ± 0.39</td>
<td>1.14 ± 0.36</td>
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<tr>
<td>LDL (mmol/L)</td>
<td>4.15 ± 0.79</td>
<td>4.05 ± 0.70</td>
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<tr>
<td>HDL (mmol/L)</td>
<td>1.61 ± 0.50</td>
<td>1.53 ± 0.30</td>
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<tr>
<td>Dietary carbohydrates (%)</td>
<td>47.1 ± 6.4</td>
<td>45.4 ± 10.2</td>
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<tr>
<td>Dietary fat (%)</td>
<td>36.4 ± 6.2</td>
<td>36.7 ± 7.2</td>
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<tr>
<td>Dietary protein (%)</td>
<td>16.5 ± 3.3</td>
<td>17.9 ± 3.5</td>
</tr>
<tr>
<td>Dietary saturated fat (%)</td>
<td>17.3 ± 5.3</td>
<td>17.2 ± 5.8</td>
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<tr>
<td>Dietary cholesterol (g)</td>
<td>0.33 ± 0.04</td>
<td>0.26 ± 0.09</td>
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<tr>
<td>Dietary n-3 fatty acids (%)</td>
<td>0.68 ± 0.24</td>
<td>0.76 ± 0.24</td>
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<tr>
<td>Dietary n-6 fatty acids (%)</td>
<td>4.30 ± 1.48</td>
<td>4.05 ± 1.07</td>
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RSX:Kacerovsky-Bielesz and Associates
Genetic modification of ATP turnover

Table 2—Exercise performance, myocellular energy metabolism, and ectopic fat contents before (baseline) and after 26 weeks of training

<table>
<thead>
<tr>
<th>Variables</th>
<th>Endurance training</th>
<th>Resistance training</th>
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<tbody>
<tr>
<td>Wkg&lt;sub&gt;max&lt;/sub&gt; (W · kg&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>2.23 ± 0.46</td>
<td>2.56 ± 0.83</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2max&lt;/sub&gt; (mL · kg&lt;sup&gt;−1&lt;/sup&gt; · min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>29.6 ± 5.70</td>
<td>31.2 ± 7.1</td>
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<tr>
<td>MaxT&lt;sub&gt;ext&lt;/sub&gt;30 (Nm)</td>
<td>342 ± 128</td>
<td>322 ± 92</td>
</tr>
<tr>
<td>MaxT&lt;sub&gt;ext&lt;/sub&gt;60 (Nm)</td>
<td>296 ± 121</td>
<td>265 ± 87</td>
</tr>
<tr>
<td>MaxT&lt;sub&gt;flex&lt;/sub&gt;60 (Nm)</td>
<td>183 ± 81</td>
<td>163 ± 42</td>
</tr>
<tr>
<td>MaxT&lt;sub&gt;flex&lt;/sub&gt;30 (Nm)</td>
<td>149 ± 60</td>
<td>122 ± 5.0</td>
</tr>
<tr>
<td>fATP (µmol · mL&lt;sup&gt;−1&lt;/sup&gt; · min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>11.6 ± 2.7</td>
<td>12.0 ± 2.4</td>
</tr>
<tr>
<td>PCr flux (µmol · mL&lt;sup&gt;−1&lt;/sup&gt; · min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>368 ± 48</td>
<td>444 ± 80</td>
</tr>
<tr>
<td>PME (mmol/L muscle)</td>
<td>0.10 ± 0.07</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>IMCL&lt;sub&gt;sol&lt;/sub&gt; (% water signal)</td>
<td>1.19 ± 0.46</td>
<td>1.06 ± 0.21</td>
</tr>
<tr>
<td>IMCL&lt;sub&gt;tib&lt;/sub&gt; (% water signal)</td>
<td>0.28 ± 0.16</td>
<td>0.31 ± 0.18</td>
</tr>
<tr>
<td>HCL (% water signal)</td>
<td>6.71 ± 9.76</td>
<td>4.37 ± 5.9</td>
</tr>
</tbody>
</table>

Data are means ± SD for endurance and resistance training groups. HCL, ectopic lipid content in liver; IMCL<sub>sol</sub>, ectopic lipid content in m. soleus; IMCL<sub>tib</sub>, ectopic lipid content in m. tibialis anterior; MaxT<sub>ext</sub>30, maximum torque of knee extensors (as the sum of the right and left side; velocity 30°/s); MaxT<sub>ext</sub>60, maximum torque of knee extensors (as the sum of the right and left side; velocity 60°/s); MaxT<sub>flex</sub>30, maximum torque of knee flexors (as the sum of the right and left side; velocity 30°/s); MaxT<sub>flex</sub>60, maximum torque of knee flexors (as the sum of the right and left side; velocity 60°/s); NS, not significant; Nm, Newton meter; PCr flux, myocellular phosphocreatine flux; PME, myocellular phosphonesters; VO<sub>2max</sub>, maximum oxygen uptake; Wkg<sub>max</sub>, maximum power.

After-training analyses
The volunteers completed 91 ± 3% of the endurance and 90 ± 3% of the resistance training sessions and responded to the respective training interventions (Table 2 and Supplementary Fig. 3). In endurance training, only watt per kg body weight at the respiratory compensation point (Wkg<sub>RCP</sub>) and VO<sub>2</sub>RCP fell (P < 0.001) by 44 and 30%, respectively. In resistance training, only MaxT<sub>ext</sub> and MaxT<sub>flex</sub> increased (P < 0.001) by 23 and 40%, respectively. Fasting plasma glucose, lipids, insulin sensitivity, and secretion did not change after endurance and resistance training (Table 1). During the OGGT, glucose and insulin concentrations were similar (Fig. 1). Adiponectin also remained unchanged in endurance and resistance training (13.10 ± 9.22 vs. 11.24 ± 7.51 µg/mL). fATP and IMCL remained unchanged in both groups (Table 2). Mean HCL tended to be, but was not, significantly lower after endurance and resistance training. Thyroid function neither changed in both groups nor correlated with insulin sensitivity or fATP across groups and within subgroups.

Post hoc subgroup analyses
As in our previous short-term exercise training study (8), participants also were analyzed according to the fATP difference between baseline and 26 weeks into responders (four from the endurance training group and four from the resistance training group), who had to show a positive increment in fATP after exercise training, and nonresponders (four from the endurance training group and three from the resistance training group) (Fig. 2). In responders, fATP increased by 26% (P < 0.05), whereas fATP even tended to decrease in nonresponders after 26 weeks. Baseline characteristics between these subgroups were identical except for the slightly older age of the responders (Supplementary Table 1).

Exercise training improved Wkg and MaxT<sub>flex</sub>60 in both subgroups but increased VO<sub>2</sub> only in responders (Supplementary Table 2). Responders slightly decreased their BMI by 2.7% without changes in WHR. During the OGGT, glucose and insulin concentrations were similar before and after exercise training (Fig. 1).

The presence of the A allele of the NDUFB6 gene polymorphism, rs540467, was associated with resistance to stimulation of fATP. After the training period, 86% of the G/G carriers (six responders and one nonresponder) but only 25% of the A allele carriers increased their fATP (P = 0.019 for a dominant model). In G/G carriers of rs540467, fATP rose by 24% (9.5 ± 2.3 vs. 11.9 ± 2.4 µmol · mL<sup>−1</sup> · min<sup>−1</sup>; P = 0.001), whereas fATP was not different in A allele carriers (13.6 ± 3.2 vs. 12.1 ± 3.4 µmol · mL<sup>−1</sup> · min<sup>−1</sup>; P = 0.18). Of all responders, 75% (six of eight) were A allele carriers of rs540467, but only 25% of the G/G carriers (six responders and one nonresponder) but only 25% of the A allele carriers increased their fATP (P = 0.019 for a dominant model). In G/G carriers of rs540467, fATP rose by 24% (9.5 ± 2.3 vs. 11.9 ± 2.4 µmol · mL<sup>−1</sup> · min<sup>−1</sup>; P = 0.001), whereas fATP was not different in A allele carriers (13.6 ± 3.2 vs. 12.1 ± 3.4 µmol · mL<sup>−1</sup> · min<sup>−1</sup>; P = 0.18).

CONCLUSIONS—This moderate exercise training program increased the respective exercise capacities with a high degree of compliance in both training groups. Despite no overall improvement of insulin sensitivity and β-cell function across all first-degree relatives, one subgroup (responders) exhibited greater muscular fATP after 26 weeks, which related to the presence of the G allele in the NDUFB6 gene.

This study unmasks a dissociation between the effects of long-term exercise training on fATP and insulin sensitivity and extends our previous observation that the G allele, but not the A allele, in the NDUFB6 gene associates with increases in both fATP and insulin sensitivity after short-term high-intensity exercise training (8). This single nucleotide polymorphism in the NDUFB6 gene nominally associates with risk markers of type 2 diabetes (9), a disorder with impaired insulin-stimulated fATP in skeletal muscle (3,4). Moreover, the function of NDUFB6 is subject to epigenetic regulation by age in human skeletal muscle and by high-fat diet in rat adipose tissue, both of which also impair mitochondrial function (9). These and the current studies suggest that the response to chronic exercise training is independently modulated by mitochondrial function and insulin sensitivity. Inherited factors at least partly control this response and may thereby contribute to the success of physical activity in relatives of patients with type 2 diabetes.

A dissociation between the stimulation of oxidative capacity, mitochondrial function, and insulin sensitivity has been reported recently. Aerobic training for 10 weeks increased oxidative capacity and...
muscle citrate synthase activity independently of changes in insulin sensitivity in obese humans with or without type 2 diabetes (20). This group further reported similar intrinsic mitochondrial respiration at lower insulin sensitivity in men with type 2 diabetes despite improved insulin resistance after 10 weeks of aerobic training (21). On the other hand, 9 days of intensive exercise training increased fATP and citrate synthase but not whole-body insulin sensitivity in relatives of mothers with type 2 diabetes (22). Likewise, increased mitochondrial capacity also is present in severely insulin-resistant Asian Indians (23), suggesting inherited variability of myocellular mitochondrial function, which is not necessarily coupled to insulin sensitivity.

In the current study, the responders were slightly older and had moderately greater WHR after the training period compared with the nonresponders, but they were not different in any other measured variable. Responders exhibited only nonsignificant trends toward lower resting fATP and higher HCL, independent of insulin sensitivity and likely corresponding to their older age. Because increasing age and body fatness can be expected to rather reduce the responses of fATP, the finding of increased fATP after exercise training is unlikely to be from nongenetic causes.

Despite successful improvement of the specifically trained exercise capacities after 26 weeks, we could not support our hypothesis that endurance training is superior to resistance training in improving insulin sensitivity and fATP. In line with previous data, circulating adiponectin also did not change during exercise training (24). Of note, VO$_{2max}$ increased significantly only in responders, suggesting that the nonresponders’ inability to raise VO$_{2max}$ despite improved maximum power output reflects their inability to increase fATP upon exercise training.

The training intensity for endurance training (80–90% of Wkg$\text{RCP}$) was chosen to allow for sufficient stimulation of both glucose and lipid oxidation, which should subsequently decrease myocellular lipid metabolites and IMCL, thereby improving insulin signaling and glucose transport/phosphorylation (i.e., insulin sensitivity) (25). Shorter-duration exercise (~115 min per week) was less effective in improving insulin sensitivity (26). Low-intensity exercise (~40% of heart rate reserve) modestly raised VO$_{2max}$ but failed to increase insulin sensitivity (27) in line with the current study. Although larger training effects have been demonstrated mainly in obese and type 2 diabetic subjects (28), both dependent (29) and independent of weight loss or fat distribution (30), less is known about their nonobese relatives. Perseghin et al. (1) reported substantially increased insulin-stimulated myocellular G6P and glycogen synthesis but no effect on glucose oxidation after 6 weeks of endurance training in first-degree relatives. The relatives of the current study showed no response in

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**Figure 1**—Blood glucose and plasma insulin concentrations during the OGTT in first-degree relatives of patients with type 2 diabetes. Means ± SD are shown for endurance (circles; n = 8) and resistance (triangles; n = 8) training groups (A) as well as for responders (squares; n = 8) and nonresponders (B) (triangles; n = 7), as defined by the difference of flux through fATP between baseline data (closed symbols) and data after 26 weeks (open symbols) of exercise training.
insulin sensitivity but evidence for greater oxidative metabolism in a subgroup, which could be attributed to their lower degree of insulin resistance. In overweight daughters of patients with type 2 diabetes, 7 weeks of endurance training increased the OGTT-derived insulin sensitivity index more than in female subjects without a family history of type 2 diabetes (31). However, OGTTs were performed only 15–24 h after the last exercise bout, indicating acute rather than sustained exercise effects. Finally, subclinical hypothyroidism also may attenuate exercise training–induced improvements of insulin sensitivity (7), but all participants of this study had normal thyroid function.

This study evaluated the effects of a long-term, supervised individual–regulated training, at twice weekly frequency, which is more realistic to be implemented in everyday life. Of note, twice-weekly training frequency was shown to improve insulin resistance using either resistance training in older men with type 2 diabetes (12) or endurance training in obese nondiabetic women (13). On the other hand (1), recent studies found no change in insulin-mediated glucose uptake but increased muscular citrate synthase activity after intensive exercise training for 9 days (22) or 12 weeks (32). The participants of the latter study were older, more obese, and had lower insulin secretion capacity compared with the relatives in our study. In younger relatives, insulin sensitivity also dissociated from \( V_{O2\max} \) responses after 10 weeks of exercise training (33). After intensive endurance training for 6 weeks, young first-degree relatives

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**Figure 2**—Skeletal muscle flux through fATP (A) and insulin sensitivity (OGIS) (B) in first-degree relatives of patients with type 2 diabetes. The graphs show the means and the individual data of responders (subjects 1–8) or nonresponders (subjects 9–15) with regard to increased fATP after 26 weeks of training. The \( P \) values are given for the comparison of data at baseline versus 26 weeks of training. NS, not significant. Subject number and training group: subject 1: endurance training; subject 2: endurance training; subject 3: endurance training; subject 4: endurance training; subject 5: resistance training; subject 6: resistance training; subject 7: resistance training; subject 8: resistance training; subject 9: endurance training; subject 10: endurance training; subject 11: endurance training; subject 12: endurance training; subject 13: resistance training; subject 14: resistance training; and subject 15: resistance training.
showed a similar incremental improvement of whole-body insulin sensitivity, along with greater muscular insulin-stimulated glucose transport/phosphorylation, but failed to normalize insulin-stimulated muscular glycogen synthesis compared with matched control subjects (1). Even patients with overt type 2 diabetes can normalize their muscular mitochondrial capacity upon 12 weeks of exercise training and still have lower insulin-stimulated nonoxidative glucose disposal than nondiabetic subjects (34). Finally, the HERITAGE study provided evidence that even 20 weeks of exercise training only gradually raised insulin sensitivity, with a broad variability and a surprisingly low rate of ~42% of responders regarding insulin sensitivity (28). Taken together, these data suggest the existence of an exercise resistance in insulin sensitivity, with a broad variability upon 12 weeks of exercise training.

Despite the strengths of this study, such as the continuously supervised, diet-controlled intervention, noninvasive phenotyping, and meticulous exclusion of acute exercise action, possible limitations have to be addressed. The frequent-sampling OGTT was used instead of the hyperinsulinemic-euglycemic clamp, which is considered the gold standard for measuring insulin sensitivity. Nonetheless, OGTTs have repeatedly shown that local perfusion, muscle fiber composition, and myocellular concentrations of triglycerides and lipid metabolites, also may be involved. The authors thank Maria Svensson, Department of Clinical Sciences, Lund University, Malmö, Sweden, for extracting DNA and Unlilec Poschen, Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research, Düsseldorf, Germany, for measuring adiponectin.

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