Genetic Variations in PPARD and PPARGC1A Determine Mitochondrial Function and Change in Aerobic Physical Fitness and Insulin Sensitivity during Lifestyle Intervention

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Context: Mitochondrial function is associated with aerobic physical fitness and insulin sensitivity and may play an important role in the pathophysiology of type 2 diabetes. Peroxisome proliferator-activated receptor (PPAR)-β (gene PPARD) and PPARγ coactivator 1α (gene PPARGC1A) are determinants of mitochondrial function in animals and in vitro.

Objective: The objective of this study was to establish whether single-nucleotide polymorphisms (SNPs) in PPARD and PPARGC1A modulate the effect of exercise training on change in aerobic physical fitness and insulin sensitivity and whether they affect mitochondrial function in human myotubes in vitro.

Setting: The study setting was the Tuebingen Lifestyle Intervention Program in a university teaching hospital.

Results: After 9 months of intervention, the minor G allele of SNP rs2267668 in PPARD and the minor serine-encoding allele of the common Gly482Ser SNP in PPARGC1A were independently associated with less increase in individual anaerobic threshold (n = 136, P = 0.002 and P = 0.005), a precise measurement of aerobic physical fitness. Moreover, individual anaerobic threshold (+11%) and insulin sensitivity (+4%) increased less in subjects carrying the minor alleles at both SNPs (X/G-X/Ser), compared with homozygous carriers of the major alleles (AA-Gly/Gly, +120% and +40%; P < 0.0001 and P = 0.015), suggesting an additive effect of the SNPs. In addition, low skeletal muscle mitochondrial function in vitro was detected in young carriers of the G allele of the SNP rs2267668 in PPARD (n = 19, P = 0.02).

Conclusions: These data provide evidence that the rs2267668 A/G SNP in PPARD and the Gly482Ser SNP in PPARGC1A have both independent and additive effects on the effectiveness of aerobic exercise training to increase aerobic physical fitness and insulin sensitivity. (J Clin Endocrinol Metab 92: 1827–1833, 2007)
polymorphisms (SNPs) in PPARD were found to be associated with skeletal muscle insulin-stimulated glucose uptake (17). The Gly482Ser amino acid exchange in the PGC1α gene (PPARGC1A) is associated with type 2 diabetes (18, 19) and physical fitness (20). Moreover, SNPs in PPARD and the SNP Gly482Ser were found to predict the conversion from impaired glucose tolerance to type 2 diabetes (21). Supposing that these SNPs modulate the effectiveness of aerobic exercise training to increase insulin sensitivity, then they are candidates for the prediction of the effectiveness of such an intervention. To address this hypothesis, we investigated whether these SNPs predict the response of aerobic fitness and prediabetes phenotypes to aerobic exercise training during a lifestyle intervention. To test functional relevance of the SNPs, we investigated whether they are associated with mitochondrial function in in vitro differentiated human myotubes.

Subjects and Methods

Lifestyle intervention

Participants. Subjects from the southern part of Germany participated in the ongoing Tuebingen Lifestyle Intervention Program that was initiated in 2003. This study was designed to uncover parameters that predict the effect of a lifestyle intervention with diet and moderate increase in aerobic physical activity to improve prediabetes phenotypes and to prevent type 2 diabetes. Subjects were included when they fulfilled at least one of the following criteria: a family history of type 2 diabetes, a body mass index greater than 27 kg/m², or a previous diagnosis of impaired glucose tolerance or gestational diabetes. They had measurements at baseline and after 9 months of intervention. Tests were performed after an overnight fast and only drinking of tap water was allowed. The participants were instructed to retain their regular diets and refrain from heavy exercise the day before the tests. Most data were available at baseline and after 9 months of follow-up so far. A total of 136 participants were included in the present analyses. Mitochondrial dehydrogenase activity in in vitro differentiated human myotubes was measured in nineteen subjects. Individuals were mostly sedentary and were not involved in regular exercise programs. Informed written consent was obtained from all participants and the local medical ethics committee approved the protocols.

Intervention with diet and physical activity. After the baseline measurements, individuals underwent dietary counseling and had up to 10 sessions with a dietician. Counseling was aimed to reduce body weight, intake of calories, and particularly intake of calories from fat and increase intake of fiber. Individuals were asked to perform at least 3 h of moderate sports per week. Endurance exercise (e.g., walking, swimming) was encouraged. Individuals were given a heart rate monitor (Polar, Büttemborn, Germany) to document exercise intensity and exercise volume. Interviews revealed that individuals occasionally exercised without wearing the pulse watch and that about two thirds of the exercise was documented by the participants.

Oral glucose tolerance test (OGTT). All individuals underwent a 75-g OGTT, and venous blood samples were obtained at 0, 30, 60, 90, and 120 min for determination of plasma glucose (bedside glucose analyzer, glucose-oxidase method; YSI, Yellow Springs Instruments, Yellow Springs, CO) and insulin (microparticle enzyme immunoassay; Abbott Laboratories, Tokyo, Japan). Insulin sensitivity was calculated from glucose and insulin values during the OGTT, as proposed by Matsuda and DeFronzo (22).

Body composition. Total body fat and lean body mass were measured by bioelectrical impedance (RJL, Detroit, MI).

Habitual physical activity. All individuals completed a standardized self-administered and validated questionnaire to measure physical activity, and a habitual physical activity (HPA) score was calculated (23).

Peak aerobic capacity on a cycle ergometer (VO2 peak cycle). The individuals underwent a continuous, incremental exercise test to volitional exhaustion using a cycle ergometer. The cycle ergometer test was performed on an electromagnetically braked cycle ergometer (Ergometrics 800 S; Ergoline, Bitz, Germany). Oxygen uptake was measured using a spirometer (MedGraphics System Breese Ex 3.02 A; MedGraphics, St. Paul, MN). Peak aerobic capacity was expressed as VO2 (milliliters per minute) per kilogram lean body mass.

Individual anaerobic threshold (IAT) and peak aerobic capacity on a treadmill (VO2 peak Tread). Subjects performed an incremental exercise test on a motorized treadmill (Saturn; HP-Cosmos, Traunstein, Germany) using a walking protocol. Initial walking speed was 3 km/h and was increased to 6 km/h after 3 min. The treadmill inclination was held constant at 0% during the first two stages and then increased by 2.5% every 3 min, whereas walking speed was held constant at 6 km/h. The test was terminated at exhaustion of the subjects. After every stage, capillary blood samples were taken from the hyperemized earlobe for the measurement of lactate (EBIO plus; Eppendorf, Hamburg, Germany). The time course of lactate was smoothed using a computerized spline procedure (Ergonorizer; Freiburg, Germany). The lactate threshold was defined at the lowest value of the lactate to performance ratio and describes the onset of lactate increase. The IAT was defined at a lactate concentration of 1.5 mmol/liter above the lactate threshold in the treadmill test (24). The rate of work done at the IAT was calculated according to method described by Forsazse et al. (25) and expressed in watts. The IAT indicates the critical value at which further increase in exercise intensity results in switch from aerobic to anaerobic metabolism. Oxygen uptake was measured using a spirometer (MedGraphics System Breese Ex 3.02 A; MedGraphics).

Cell culture and estimation of mitochondrial activity in vitro

Primary human skeletal muscle cells were grown from satellite cells obtained from percutaneous needle biopsies performed on the lateral portion of the quadriceps femoris (vastus lateralis) muscle. Satellite cells were obtained from the biopsies by collagenase digestion as previously described (26). Cells were seeded and expanded in a α-MEM/Ham’s F-12 (1:1) mixture supplemented with 20% fetal calf serum, 1% chicken embryo extract, and 0.2% antibiotic antymycotic solution as described (26). First-pass cells were used for experiments. After having reached 80–90% confluence, myoblasts were shifted for 5 d to α-MEM supplemented with 2% fetal calf serum and 0.2% antibiotic antymycotic solution to differentiate to myotubes. At the end of the protocol, most of the cells were fused to myotubes (27). For the estimation of mitochondrial activity, we measured the mitochondrial respiratory chain function (electron transport capacity of the complex II) as previously described (28). The cleavage of 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT) into formazan by the succinate-tetrazolium reductase system (EC 1.3.99.1), which belongs to the respiratory chain of the mitochondria, is used as an estimate for the electron transport capacity of the mitochondria. In brief, cells cultured in duplicate were incubated for 4 h with 0.5 mg/ml MTT before overnight lysis by addition of two volumes of 10% sodium dodecyl sulfate in 0.01 mol/liter HCl. The Cell Proliferation Kit I (MTT) from Roche Diagnostics (Basel, Switzerland) was used. The lysates were transferred into tubes, shaken, and then formazan dye produced by mitochondrial dehydrogenases was photometrically measured at 565 nm.

Genotyping

Three haplotype tagging SNPs in PPARD rs6690123, rs1053049, and rs2076167 that were in close linkage disequilibrium (LD) in a Finnish population (16) and the haplotype tagging SNP rs2367668 that was in less strong LD with those SNPs were genotyped. DNA was isolated from whole blood using a commercial DNA isolation kit (NucleoSpin; Macherey-Nagel, Düren, Germany). Genotyping of the SNPs in PPARD was achieved using the TaqMan assay (Applied Biosystems, Foster City, CA). The TaqMan genotyping reaction was amplified using a GeneAmp PCR system 7000 (95 C for 10 min, then 38 cycles at 95 C for 15 sec, 62 C for 1 min), and fluorescence was detected using an ABI Prism 7000 sequence detector (Applied Biosystems). The overall genotyping success
rate was 99.9%, and rescreening of 3.1% of subjects gave 100% identical results. The Gly482Ser variant in PPARGC1A was determined by bidirectional sequencing (100% concordance) as previously described (29).

**Statistical analyses**

Data are given as mean ± se. Statistical comparison was performed using logistically transformed data (for nonnormally distributed parameters). Differences between measurements at baseline and at follow-up were tested with the paired t test. Differences in changes in anthropometrics between genotypes were tested using multivariate linear regression models. In these models, fold change in the trait was the dependent variable, whereas the trait at baseline, age, gender, and the genotype were the independent variables. Differences in changes in metabolic characteristics were additionally adjusted for body fat at baseline and at follow-up. Pairwise LD and haplotypes were determined using the THESIAS program (30). THESIAS performs haplotype-based association analyses in unrelated individuals with phase-unknown genotype data. The simultaneous estimation of haplotype frequencies and of their associated effects on the phenotype of interest.

**Results**

**Screening for polymorphisms and genotyping results**

Four SNPs in PPARD (rs6902123, rs2267668, rs2076167, and rs1053049) and the Gly482Ser variant in PPARGC1A were genotyped. All allele frequencies were in Hardy–Weinberg equilibrium (χ² test, P > 0.05). The minor allele frequencies were 0.10 (C allele) for rs6902123, 0.17 (G for rs2267668), 0.25 (C for rs2076167), 0.25 (C for rs1053049), and 0.32 for the Ser482-encoding allele. The SNPs in PPARD were in strong pairwise LD (all D² > 0.80, P < 0.02) and the SNPs rs2076167 and rs1053049 were in complete LD.

**Univariate relationships**

At baseline insulin sensitivity was positively correlated with VO₂peak_cycle (r = 0.32, P = 0.0002), VO₂peak_TM (r = 0.18, P = 0.04), IAT (r = 0.20, P = 0.02), and, although this was statistically not significant, with HPA score (r = 0.16, P = 0.07). IAT was strongly correlated with VO₂peak_cycle (r = 0.69, P < 0.0001), VO₂peak_TM (r = 0.50, <0.0001) but not with HPA score (r = 0.14, P = 0.11). VO₂peak_cycle and VO₂peak_TM were strongly correlated (r = 0.52, P < 0.0001) and VO₂peak_TM (r = 0.19, P = 0.04) but not VO₂peak_cycle (r = 0.16, P = 0.10) was correlated with HPA score.

**Change in parameters during the lifestyle intervention**

Table 1 presents the demographic and metabolic characteristics at baseline and at follow-up. The population consisted of 63 males and 73 females with a mean age of 45 yr (range 19–67). During a follow-up of 9 months, body weight decreased by 3% and body fat by 6%. Except for fasting glucose levels, fasting insulinemia as well as 2 h glyceremia and insulinemia decreased significantly. In addition, a mean increase in insulin sensitivity by 14% was observed.

The HPA score, representing the average daily physical activity, also increased significantly and a mean (±se) daily aerobic exercise volume of 19 ± 1.9 min was documented by the participants with the pulse watch. This resulted in a mean increase in the IAT by 22%, the VO₂peak_TM by 6%, the VO₂ IAT TM by 6%, and the VO₂ peak cycle by 3%.

**Genetic effects on changes in the parameters during the lifestyle intervention**

Because the SNPs rs1053049 and rs2076167 were in complete LD, only data for the SNP rs1053049 are shown. Subjects carrying the C allele of SNP rs1053049 in PPARD had less decrease in fasting insulin levels (P = 0.01) and less increase in insulin sensitivity (P = 0.047), compared with subjects with the T/T genotype (Table 2). Insulin sensitivity (P = 0.041) and IAT (P = 0.003) increased less, and VO₂peak_cycle (P = 0.048) even decreased in carriers of the G allele of SNP rs2267668, compared with subjects with the A/A genotype. For the Gly482Ser polymorphism in PPARGC1A, less increase in IAT was observed in carriers of the Ser482-encoding allele, compared with the Gly/Gly genotype (P = 0.004). The increase in insulin sensitivity was also smaller in carriers of the Ser482-encoding allele but, however, did not reach statistical significance (P = 0.12).

Changes in fasting insulinemia for the SNP rs1053049, IAT for SNP rs2267668, and the Gly482Ser polymorphism re-

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**TABLE 1.** Characteristics of 136 subjects who underwent the lifestyle intervention

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
<th>P</th>
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<tbody>
<tr>
<td>Demographics</td>
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<td></td>
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<tr>
<td>Gender (men/women)</td>
<td>63/73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>45 ± 1</td>
<td></td>
<td></td>
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<tr>
<td>Body weight (kg)</td>
<td>86.6 ± 1.5</td>
<td>83.8 ± 1.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>31.0 ± 0.7</td>
<td>29.2 ± 0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Metabolic characteristics</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fasting glucose (mM)</td>
<td>5.21 ± 0.10</td>
<td>5.16 ± 0.04</td>
<td>0.08</td>
</tr>
<tr>
<td>2-h glucose (mM)</td>
<td>7.07 ± 0.14</td>
<td>6.46 ± 0.13</td>
<td>&lt;0.0001</td>
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<tr>
<td>Fasting insulin (pm)</td>
<td>60 ± 3</td>
<td>52 ± 3</td>
<td>0.0007</td>
</tr>
<tr>
<td>2-h insulin (pm)</td>
<td>481 ± 34</td>
<td>392 ± 31</td>
<td>&lt;0.0001</td>
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<tr>
<td>Insulin sensitivity (OGTT) (arbitrary units)</td>
<td>13.37 ± 0.62</td>
<td>15.27 ± 10.68</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HPA score</td>
<td>8.18 ± 0.10</td>
<td>8.64 ± 0.08</td>
<td>&lt;0.0001</td>
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<tr>
<td>IAT (W)</td>
<td>77 ± 4</td>
<td>94 ± 4</td>
<td>&lt;0.0001</td>
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<tr>
<td>VO₂peak_TM (ml·min⁻¹·kg·lbm⁻¹)</td>
<td>43 ± 1</td>
<td>45 ± 1</td>
<td>0.0001</td>
</tr>
<tr>
<td>VO₂IAT peak_TM (ml·min⁻¹·kg·lbm⁻¹) (% of VO₂peak)</td>
<td>25 ± 1 (59 ± 1)</td>
<td>27 ± 1 (62 ± 1)</td>
<td>0.0008</td>
</tr>
<tr>
<td>VO₂peak_cycle (ml·min⁻¹·kg·lbm⁻¹)</td>
<td>37 ± 1</td>
<td>38 ± 1</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data represent mean ± se. HPA, n = 128. VO₂ values during the treadmill (TM) test were available in 128 and during the cycle ergometer test in 125 subjects. lbm, Lean body mass.
TABLE 2. Associations of the SNPs in PPARD and PPARGC1A with changes in adiposity and metabolic characteristics during lifestyle intervention

| SNP    | Gender (men/women) | Age (yr) | Body weight (kg) | Body fat (%) | Fasting glucose (mmol/l) | 2h glucose (mmol/l) | Baseline insulin (pM) | HPA score | Data are mean ± SD. HPA,0.5 = 128. VO2 values during the treadmill (TM) test were available in 128 and during the cycle ergometer test in 125 subjects. For statistical analyses, all changes in the parameters were adjusted for values at baseline. VO2peak was additionally adjusted for age and gender. All other changes were adjusted for age, gender, and body fat at baseline and at follow-up. lbm, Lean body mass.

- **PPARD**
  - Gly482Ser
  - Gly62
  - rs6902123
  - rs1053049

- **PPARGC1A**
  - rs2267668
  - rs2076167

**Independent effects of the SNPs**

Because the SNP rs2267668 in PPARD and the SNP Gly482Ser in PPARGC1A had large effects on IAT, we tested whether the relationships of the SNPs with change in IAT were independent of each other. In a multivariate regression model less increase in IAT was predicted by both SNPs (P = 0.002 for SNP rs2267668 and P = 0.003 for SNP Gly482Ser). Using a similar model with insulin sensitivity instead of IAT, less increase in insulin sensitivity was found for the SNP rs2267668 (P = 0.03), whereas the effect of the SNP Gly482Ser did not reach statistical significance (P = 0.11). This suggests that the effects of the SNP’s on change in IAT are independent of each other. An independent effect was found for the SNP rs2267668 in PPARD for change in insulin sensitivity.

**Additive effects of the SNPs**

To determine further whether the SNPs have an additive effect on IAT and insulin sensitivity, genotype interactions were tested. Among the four genotype groups (A/A-Gly/Gly, X/G-Gly/Gly, A/A-X/Ser, and X/G-X/Ser), there was an overall significant genotype effect for change in IAT (ANOVA, P = 0.0003; Fig. 1A) and a trend for change in insulin sensitivity (P = 0.08; Fig. 1B). Subjects carrying both at-risk alleles (X/G-X/Ser, n = 20) had less increase in IAT (11%) and less increase in insulin sensitivity (4%), compared with subjects who were homozygous for both protective alleles (A/A-Gly/Gly, n = 44, 120%, P < 0.0001, and 40%, P = 0.015; new alpha level 0.0127 after adjustment for multiple comparisons for four genotype combinations; Fig. 1, A and B). The results were similar after additional adjustment for the HPA scores at baseline and at follow-up and the documented exercise volume (n = 128, P = 0.006 and P = 0.0001 for change in IAT; P = 0.04 and P = 0.03 for change in insulin sensitivity) as well as after adjustment for dietary intake (data not shown).

Furthermore, compared with homozygous carriers of both protective alleles (A/A-Gly/Gly), carriers of both at-risk alleles (X/G-X/Ser) had a 10.1-fold higher risk (95% confidence interval 2.49–52.05, P = 0.003) not to respond with a greater than 10% increase in IAT and a 9.3-fold higher risk (95% confidence interval 2.28–49.89, P = 0.004) not to respond with a greater than 10% increase in insulin sensitivity during the intervention, independently of the covariates.

tained significance after adjustment for multiple comparisons for four SNPs at the new alpha level 0.0127.

**Haplotype analyses**

Based on the four SNPs in PPARD, six haplotypes were found. Compared with the most common haplotype carrying the major alleles in all SNPs (TTAT; rs1053049, rs6902123, rs2267668, rs2076167; frequency: 0.72), the haplotype CTGC (allele frequency 0.14) was associated with less increase in IAT (P = 0.0002) and less increase in insulin sensitivity (P = 0.03), independent of covariates. Change in IAT was still significantly after adjustment for multiple comparisons for six haplotypes at the new alpha level 0.0085.
Mitochondrial activity of myotubes

To determine the functional relevance of the SNPs in PPARD and the SNP Gly482Ser in PPARGC1A on mitochondrial activity, we performed an MTT assay to determine mitochondrial dehydrogenase activity in cultured myotubes. Gender distribution of the donors was 13 males and six females with a mean age of 26 yr, compared with the participants who underwent the lifestyle intervention (mean age 45 yr). As reported by Ling et al. (41), PGC1α mRNA expression in human skeletal muscle was lower only in elderly but not young individuals carrying the 482Ser-encoding allele, which supports our findings.

The SNP rs2267668 in PPARD was associated with aerobic physical fitness, determined by the IAT and VO2 peak cycle and with estimated mitochondrial function. A haplotype carrying the minor allele at this locus also displayed a pronounced interaction with each other to influence these factors (6–9). In addition, they may regulate not only the response of aerobic fitness to aerobic training but also prediabetes phenotypes, such as insulin sensitivity, which is commonly improved by physical activity (35–40). Whereas no data on the relationship of SNPs in PPARD with physical fitness exist, the frequency of the 482Ser-encoding allele in PPARGC1A was lower in endurance-trained male athletes, compared with the participants who underwent the lifestyle intervention (mean age 45 yr). As reported by Ling et al. (41), PGC1α mRNA expression in human skeletal muscle was lower only in elderly but not young individuals carrying the 482Ser-encoding allele, which supports our findings.

Discussion

In the present study, we provide novel information that SNPs in PPARGC1A and PPARD are associated with the effectiveness of aerobic exercise training to increase aerobic fitness and with change in insulin sensitivity during a lifestyle intervention. Moreover, we found that a SNP in PPARD is a determinant of the estimated mitochondrial function in vitro.

Lifestyle interventions were found to be effective for reducing the relative risk of type 2 diabetes (31–36), and exercise was identified as a major contributor to this beneficial effect (32, 37). More recently it was confirmed that aerobic training with dietary restriction in sedentary obese adults resulted in enlargement of mitochondria and an increase in the mitochondrial content in skeletal muscle. This was accompanied by improvements in insulin sensitivity (38). However, there is a large variability in the outcome of such interventions, and some subjects have only a minor profit. An explanation may be hereditary lower oxidative capacity due to mitochondrial dysfunction and less type I fibers in skeletal muscle of these individuals, resulting in less energy transduction. PGC1α and PPARβ are major candidates that interact with each other to influence these factors (6–9). In addition, they may regulate not only the response of aerobic fitness to aerobic training but also prediabetes phenotypes, such as insulin sensitivity, which is commonly improved by physical activity (35–40). Whereas no data on the relationship of SNPs in PPARD with physical fitness exist, the frequency of the 482Ser-encoding allele in PPARGC1A was lower in endurance-trained male athletes, compared with the participants who underwent the lifestyle intervention (mean age 45 yr). As reported by Ling et al. (41), PGC1α mRNA expression in human skeletal muscle was lower only in elderly but not young individuals carrying the 482Ser-encoding allele, which supports our findings.

The SNP rs2267668 in PPARD was associated with aerobic physical fitness, determined by the IAT and VO2 peak cycle and with estimated mitochondrial function. A haplotype carrying the minor allele at this locus also displayed a pronounced effect on IAT. Furthermore, the effects were independent of the Gly482Ser SNP in PPARGC1A, supporting the role of the SNP rs2267668 in PPARD in the modulation of aerobic fitness. Whereas the effects were found with the IAT, and to a lesser extent with VO2 peak cycle, VO2 peak TM and VO2 IAT TM did not display associations with the SNPs. An explanation...
for these differences is not easily obvious. Because oxygen uptake is also influenced by the cardiorespiratory system (42), our data imply that estimated mitochondrial function may be more closely reflected by the IAT than the oxygen uptake.

Having found that the SNPs had independent effects on IAT, we further tested whether these effects were additive. In fact, subjects carrying the minor alleles of both SNPs had the least increase in IAT. Moreover, these individuals also had the least increase in insulin sensitivity. The genetic distribution further revealed that the increase in IAT was less in subjects carrying the minor 482Ser allele of the SNP in PPARGC1A than in subjects carrying the minor allele of the SNP rs2267668 in PPARD. This may suggest that PGC1α was a stronger regulator of mitochondrial function than PPARα. However, no difference between the effects of these alleles was found for insulin sensitivity, possibly due to the smaller increase in this parameter. Ultimately, our sample size is too small to address this hypothesis fully.

Our data on the role of genetic variability in PPARD in the determination of prediabetes phenotypes are further supported by a recent report from the STOP-NIDDM trial. In that study the rare alleles of SNPs in PPARD that are in LD with the SNP rs2267668 and the 482Ser-encoding allele of PPARGC1A had additive effects on the risk of type 2 diabetes (21).

It is of note that impaired insulin signaling also plays an important role in the development of mitochondrial dysfunction (43). Because PPARα and PGC1α are involved in the regulation of insulin sensitivity, it may well be that the SNPs are associated with altered insulin sensitivity and secondarily with mitochondrial function.

A limitation of our study was that we did not directly measure ATP generation in our in vitro experiments. The cleavage of MTT into formazan by the succinate-tetrazolium reductase system, which belongs to the respiratory chain of the mitochondria, is a measurement of the electron transport capacity of the mitochondria and thus serves as an estimate of mitochondrial activity. Also, multiple comparison may be an issue. Our a priori hypothesis in this study, which was based on data in the literature, was that these SNPs may affect changes in aerobic fitness and insulin sensitivity. This naturally results in performing a large number of tests with an increased risk for a type 1 error. Although the genotypes and phenotypes tested were located where highly correlated, we performed Bonferroni corrections. It turned out that most of the major findings retained statistical significance.

So far, there is no replication of the relationships between the SNPs in PPARGC1A and PPARD with change in aerobic physical fitness. With the IAT, we had a precise measurement to detect effects on aerobic fitness in a short time of follow-up in a relatively small group of subjects. Larger intervention studies with measurements of aerobic fitness need to further corroborate the role of genetic variability in PPARGC1A and PPARD in coupling aerobic exercise with glucose and lipid metabolism.

In conclusion, these data provide evidence that the rs2267668 G/A SNP in PPARD and the Gly482Ser SNP in PPARGC1A have an independent and an additive impact on the effectiveness of aerobic exercise training to increase aerobic physical fitness and insulin sensitivity. Thus, they are candidates for the prediction of the effectiveness of such an intervention. Characterization of populations for these SNPs and the definition of an individual genetic risk score may be helpful to early implement alternative strategies such as more intense dietary modifications or pharmacological intervention.

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References


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