Short-term exercise training does not stimulate skeletal muscle ATP synthesis in relatives of humans with type 2 diabetes

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Background. We tested the hypothesis that short-term exercise training improves hereditary insulin resistance by stimulating ATP synthesis and investigated associations with gene polymorphisms.

Methods. 24 nonobese first-degree relatives of type-2 diabetic patients and 12 controls were studied at rest and 48 hours after three bouts of exercise. In addition to measurements of oxygen uptake and insulin sensitivity (OGTT), ectopic lipids and mitochondrial ATP synthesis were assessed using $^1$H and $^{31}$P magnetic resonance spectroscopy (MRS), respectively. They were genotyped for polymorphisms in genes regulating mitochondrial function, PPARGC1A (rs8192678) and NDUFB6 (rs540467).

Results. Relatives had slightly lower ($p=0.012$) insulin sensitivity than controls. In controls, ATP synthase flux rose by 18% ($p=0.0001$) being 23% higher ($p=0.002$) than in relatives after exercise training. Relatives responding to exercise training with increased ATP synthesis (+19%, $p=0.009$) showed improved insulin sensitivity ($p=0.009$), whereas “non-responders” failed to increase their insulin sensitivity. A polymorphism in the NDUFB6 gene from respiratory-chain complex I related to ATP synthesis ($p=0.02$) and insulin sensitivity response to exercise training ($p=0.05$) ATP synthase flux correlated with O$_2$ uptake and insulin sensitivity.

Conclusions. The ability of short-term exercise to stimulate ATP production distinguished individuals with improved insulin sensitivity from those who did not improve their insulin sensitivity. In addition, the NDUFB6 gene polymorphism appeared to modulate this adaptation. This suggests that genes involved in mitochondrial function contribute to the response of ATP synthesis to exercise training. This trial has been registered at ClinicalTrials.gov (NCT 00710008).
Life style intervention is the recommended strategy for prevention of type 2 diabetes mellitus (T2DM). First-degree relatives of patients with T2DM (REL) have an increased risk of insulin resistance and T2DM (1, 2). Inherited and environmental factors cause insulin resistance via intracellular lipid and inflammatory mediators which interfere with insulin signaling leading to an impaired rise of glucose-6-phosphate (G6P) due to reduced glucose transport/phosphorylation (2, 3). These alterations can coexist with excessive storage of intramyocellular or hepatocellular lipids (IMCL, HCL) and impaired mitochondrial function and/or number in insulin resistant states such as aging (4), free fatty acids (FFA) elevation (5) and in some (6-8) but not all (9, 10) humans at risk of or with T2DM. Non-diabetic REL suffering from severe insulin resistance present with elevated FFA, IMCL and HCL along with impaired ATP synthesis possibly due to reduced mitochondrial contents (11). Inherited and acquired factors associate with gene expression of the respiratory chain components, NDUFB6 and COX7A1, and their transcriptional co-activators, PGC-1α/β, which determine maximum oxygen uptake (VO₂max) and insulin action (12, 13). It remains unclear whether altered ATP synthesis results from increased availability of lipid and/or adipokines such as adiponectin, the nicotinamide phosphoribosyltransferase, visfatin, and retinol binding protein-4 (RBP4) (14).

It is further uncertain whether such abnormalities are reversible by exercising and/or occur independently of effects on insulin action. Long-term endurance exercise training increases insulin sensitivity in sedentary young and elderly (15), REL (16), glucose intolerant, obese or T2DM (17). Exercise training for at least 4 weeks enhances fat oxidation along with increased mitochondrial mass and enzyme activities (18, 19). However, little is known on time course and onset of changes in glucose and energy metabolism independently of acute exercise effects.

We employed magnetic resonance spectroscopy (MRS) to measure in vivo flux of inorganic phosphate (Pi) to ATP through ATP synthase (fATPase) as well as IMCL, HCL before and after three bouts of cycling training to test the following hypotheses: (i) increased fATPase is an early event in the response to short term exercise training, (ii) responses of fATPase and insulin sensitivity are different in REL compared with healthy controls (CON), and (iii) the responses are modulated by polymorphisms in genes which are mutually linked to exercise capacity, energy metabolism and insulin sensitivity in epidemiologic studies.

METHODS

Volunteers. We recruited healthy REL of one (n=19) or two (n=5) parents with T2DM, which was confirmed by hyperglycemia, oral antidiabetic medication or insulin use. Twelve CON matched for sex, age, body mass index (BMI) and physical activity were recruited as controls (Table 1). The participants underwent medical history and physical examination. All participants were weight-stable over the last 6 months prior to the study. None of them was smoking or regularly performing intense exercise.

Study protocols. Volunteers gave written informed consent to the study which was approved by the institutional ethical board and performed according to the Declaration of Helsinki. All participants remained on an isocaloric diet, refrained from any physical exercise for three days and fasted for 12 h before the start of the studies. On day 1, participants underwent a frequent sampling 75-g OGTT and MRS. On day 2, they performed exercise testing. On days 3 and 5, they exercised on a cycling ergometer. On
day 7, measurements of day 1 were repeated in identical fashion.

Dietary assessment. Throughout the study, all volunteers were on a dietary plan reflecting isocaloric diet in line with the American Diabetes Association recommendations. Dietary intake over the last year and dietary compliance were assessed with a modified interviewer-administered 107-item food frequency questionnaire adjusted for local dietary habits (http://www.unihohenheim.de/wwwin140/inf o/interaktives/foodfreq.htm). During the one-week intervention, volunteers counted all food and beverages consumed employing common household measures to obtain six-days dietary records. Nutrient/fluid intake on the days before the studies were analyzed using 24-hour recalls. In the evenings before the studies, participants consumed identically composed carbohydrate-enriched dinners at identical times. On study days, participants did not receive any calories except for the OGTT until completion of the MRS measurements.

Genotyping. Genomic DNA was extracted from blood of all participants by QIAamp DNA Blood Mini kit (Cat. No 51106, Qiagen). Single nucleotide polymorphisms (SNPs), rs540467 of NDUFB6 and rs8192678 (Gly482Ser) of PPARGC1A, were genotyped using allelic discrimination assays performed with an ABI 7900 system (Applied Biosystems Inc.). The following assays were used for rs540467 (Assay on demand, C_2334430, Applied Biosystems Inc.) and for rs8192678, forward primer TGGAGAATTGTTCAATTACTGAAATCAC TGT, reverse primer GGTCTACACAGTCAAGCTTTT together with two different probes Vic- CAAGACCGGTGAACTG and FamACACAGCTGAACTG, respectively.

Oral glucose tolerance test (OGTT). Participants drank a solution containing 75 g of glucose and venous blood samples were collected before and in 30-min intervals for 150 min for measurements of glucose, insulin and C-peptide. Dynamic insulin sensitivity was assessed by OGTT using the 120-min formula, which yields a measure of glucose clearance that has been widely exploited and validated against whole body insulin sensitivity obtained from the euglycemic-hyperinsulinemic clamp (20). Beta cell function was assessed from the insulinogenic index (21).

Magnetic resonance spectroscopy (MRS). Participants were studied in a 3-T MR spectrometer (Bruker, Germany). A 10-cm circular double resonant $^1$H/$^3$P surface coil was used for quantifying HCL and phosphorus metabolites. A 28-cm birdcage coil was positioned over the right lower leg for measuring IMCL in soleus and tibialis anterior (ant.) muscles. For non-localized $^3$P MRS, the right calf was positioned on the surface coil with the medial head of the right gastrocnemius muscle in the coil center. The integral of the region of phosphomonoesters (PME) covering G6P (7.1-7.4 ppm), phosphodiester (PDE), Pi and phosphocreatine (PCr) were measured from the ratio of integrated peak intensities and β-ATP resonance intensity in spectra without inversion and saturation assuming an ATP concentration of 5.5 mmol/l (Figure 1). The assumption of constant ATP before and after exercise training was supported by unchanged ATP/PDE ratios (data not shown), because PDE levels remain constant under similar conditions (22). Absolute quantification would be required to detect subtle changes of myocellular ATP concentrations, which do not necessarily reflect actual flux through ATP synthase. The saturation transfer experiment (selective irradiation of $\gamma$-ATP) was employed to measure the exchange rate ($k_1$) between Pi and ATP and to calculate fATPase from $k_1x[Pi]$ (5, 8) (Figure 1). IMCL and HCL were determined within
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volumes of interest of 1.73 cm$^3$ (23) and 27 cm$^3$ (5), respectively.

**Habitual physical activity, exercise capacity and training.** Physical activity was assessed by interviewer-administered questionnaire on a scale from one to five, low to high degree of activity (24). Each participant performed an incremental exhaustive exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) at 70 revolutions/min. Respiratory gas exchange measures were determined by open-air spirometry (Jäger/Viasys MasterScreen CPX, Würzburg, Germany). The breath-by-breath measures were recorded and averaged over an interval of eight breath cycles. Heart rate was measured every 5 s (Polar Vantage NV telemetry, Polar Electro, Kempele, Finland). To regulate load intensities of training sessions according to the individual aerobic capacity, a ventilatory threshold (RCP) was determined (25), which marks the onset of hyperventilation during incremental exercise training mainly driven by the onset of lactic acidosis. RCP was determined independently by two investigators (intraclass correlation, $r=0.9721$) from: (i) the second upward inflection in $V_E$ (minute ventilation) curve following the first break point at the anaerobic threshold, (ii) an upward inflection in the curve of $V_E/VCO_2$ (ventilatory equivalent for $CO_2$) and (iii) a downward inflection in the curve of $P ET_{CO_2}$ (end-tidal volume for $CO_2$) during the time course of respiratory gas exchange measure variables. The two training sessions (day 2, 5) consisted of 30 min in three 10-min bouts separated by two 5-min breaks with the training intensity set at 90-% work load determined at RCP. Thus, the three bouts of exercise training comprised of the exercise testing and the two subsequent training sessions. All participants completed exercise testing and all training sessions which were performed under continuous supervision by a sports physiologist. Data analysis revealed variable fATPase responses to exercise training among REL compared with CON. REL were therefore divided into two subgroups based on the difference of fATPase between baseline and after exercise training: responder (RESP, n=10) with $(fATPase_{after} - fATPase_{before}) > 0$ and non-responder (NRES, n=14) with $(fATPase_{after} - fATPase_{before}) \leq 0$.

**Analytical procedures.** Plasma glucose was measured on a Glucose analyzer II (Beckman Coulter, http://www.beckman-coulter.com). For FFA measurement (Wako Chem USA Inc., http://www.wakousa.com/), blood was collected into vials containing orlistat to prevent in vitro lipolysis. Lactate was determined enzymatically (Roche, http://www.roche.com/home.html), insulin and C-peptide by double antibody RIA and, RBP-4 and visfatin in 7 controls and 8 relatives by ELISA (Phoenix Peptides, Karlsruhe, Germany, www.phoenixpeptide.com/), with inter/intraassay coefficients of variation of <6% (5).

**Statistics.** OGIS and fATPase were defined as primary endpoints. Sample size calculation was based on previous studies on fATPase using false positive ($Z_{\alpha}=1.96$, 2-tailed) and false negative error rates ($Z_{\beta}=0.84$, 1-tailed), respectively. Data are presented as means±SD and 95% confidence intervals (text, tables) or means±standard errors (figures). Unpaired/paired two-tailed t tests were used for between- and within-group comparisons as appropriate. Bivariate correlations were assessed with Pearson’s correlations coefficient. Multiple step-wise linear regression analysis was performed for the dependent variables, fATPase and OGIS, including BMI, WHR, age, triglycerides, FFA, physical activity, VO$_2$max, HCL, IMCL, caloric intake, and also for changes in the above parameters during the study. All calculations were done using SPSS for Windows (version 8; SPSS Inc., Chicago, IL http://www.spss.com). Associations between
genotypes and exercise responses for fATPase and OGIS were analyzed with the χ²-test (NCSS Statistical Software, Kaysville, UT, USA).

RESULTS

Clinical characteristics, exercise testing and dietary intake
The groups showed similar sex distribution, age, fat mass and distribution (Table 1). Lab tests, exercise performance and physical activity including its individual components (data not shown) were not different between groups and subgroups. Energy and nutrient intake were not different between groups before and during the 24-hours prior to studies (Table 2). The six-days dietary records confirmed similar nutrient composition (Supplementary Table 1). Body weights neither changed in CON (before: 71.50±12.82 vs. after: 71.35±12.75 kg) nor REL (73.46±13.54 vs. 73.06±13.22 kg).

Metabolites, hormones, insulin sensitivity and secretion. Fasting plasma glucose did not differ between groups (Table 3). The 2-hours plasma glucose after oral glucose loading was within the normal range, slightly higher in REL before (6.3±1.4 vs. CON: 5.0±1.1 mmol/l, p=0.009) but not after exercise (4.8±1.2 vs. 5.5±1.2 mmol/l). Plasma FFA were comparable at baseline, but higher in REL after training (p=0.019). C-peptide was higher in REL before (P=0.006) and after exercise training (p=0.031). Fasting plasma insulin tended to be increased in REL. In subgroups, plasma RBP-4 (before: CON: 0.36±0.12, REL: 0.41±0.07, after training 0.36±0.08, 0.43±0.09 ng/ml) and visfatin (before: CON: 39±26, REL: 29±9, after training 35±13, 26±6 ng/ml) did not differ. At baseline, OGIS was 13% lower (p=0.012) in REL than in CON and slightly rose in both CON by 7% (p=0.05) and REL by 12% (p=0.012) after training (Figure 2). QUICKI and insulinogenic index were comparable before and after training in both groups (Table 3).

In subgroups, responding (RESP) or not responding (NRES) to exercise with stimulation of fATPase, fasting glucose, lactate, insulin, insulinogenic index C-peptide (Table 3) and 2-h post-load plasma glucose (data not shown) were comparable before and after training. Plasma FFA were similar at baseline and increased after exercise training by 39% in NRES (p=0.008). OGIS was comparable at baseline, but increased only in RESP upon exercise training (P=0.009) (Figure 2).

Intramyocellular phosphorus metabolism and ectopic lipids. Baseline fATPase did not differ between groups (CON: 12.0±2.2, REL: 11.1±2.9 µmol.ml muscle⁻¹.min⁻¹) (Figure 3). After training, fATPase increased by ~18% (P=0.0001) only in CON (14.2 ±2.5 µmol.ml muscle⁻¹.min⁻¹) and was ~23% (P=0.002) higher than in REL (10.9±3.0 µmol.ml muscle⁻¹.min⁻¹) in whom it did not change upon exercise training (Figure 3). Similarly, k₁ rose in CON (p=0.001) but not in REL (p=0.7). HCL tended to be higher in REL before training (CON: 1.5±1.0%, REL: 5.8±7.5%, p=0.08) (Figure 4), but were not different after training (CON: 1.8±1.0%, REL: 4.4±5.4%). IMCL (Figure 4) and G6P (data not shown) were neither different between groups nor affected by training.

Interestingly, baseline fATPase was higher (p=0.025) in NRES than RESP before exercise training, but by definition only increased in RESP by ~24% (p=0.009) and decreased in NRES by ~16% (p<0.001). Similarly, k₁ rose in RES (p=0.01) and decreased in NRES (p=0.02). HCL and IMCL in soleus muscle were comparable before and after the two training sessions. Baseline IMCL in tibialis anterior muscle was similar, but decreased by 35% (p=0.006) in NRES after training (data not shown).

Baseline fATPase related positively to VO₂ (r=0.300, p=0.046) and power output
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(r=0.361, p=0.030) each at maximum and RCP. This also held true for fATPase after exercise training (data not shown). Baseline fATPase correlated positively with OGIS (r=0.360, p=0.031). HCL related negatively to OGIS (r=-0.675, p<0.001) and positively to plasma insulin (r=0.761, p<0.001). Multiple regression analysis revealed that HCL and baseline caloric intake explained ~49% of the variation of baseline OGIS (p=10^{-5}). Changes in fATPase (before vs. after exercise sessions) were neither related to changes in OGIS (r=0.001, p=0.993) nor to changes in caloric intake (before vs. mean caloric intake during the intervention week; r=0.078, p=0.665) which did not relate to each other, respectively (r=-0.201, p=0.261). Multiple regression analysis did not identify any anthropometric, dietary or lab parameters as independent predictors of the post-exercise changes in fATPase.

Associations between SNPs in the NDUFB6 and PPARGC1A genes and exercise responses of fATPase and insulin sensitivity. The NDUFB6 SNP, rs540467 G/A, was associated with resistance to stimulation of fATPase and OGIS upon exercise training (Table 4). Exercise training increased fATPase in 74% of the G/G carriers, but only in 33% carrying the A-allele (p=0.02 for a dominant model). More G/G genotype carriers (84%) increased OGIS after exercise training compared with A-allele carriers (53%, p=0.05 for a dominant model). The PPARGC1A SNP (rs8192678, Gly482Ser) neither related to fATPase nor OGIS responses to exercise training (Table 4).

DISCUSSION

At baseline, REL and CON did not differ in parameters commonly interfering with insulin sensitivity and fATPase including age, fat mass, physical activity and aerobic capacity. Nevertheless, REL were slightly less insulin sensitive based on OGIS which corresponds to M-values of ~10 mg.kg\(^{-1}\).min\(^{-1}\). The small difference in OGIS is in line with the variability of insulin sensitivity in overweight (26), healthy humans and T2DM relatives (16) obtained with clamp tests. Severely insulin-resistant offspring of T2DM exhibit increases in plasma FFA, body fat mass, IMCL and HCL along with impaired insulin-stimulated glucose transport/phosphorylation (11). Such individuals have ~30% lower fATPase along with ~40% lower muscle mitochondrial density than healthy humans. In our study, fATPase was similar to that of the present and previous controls (4, 5, 8) suggesting that their ATP production suffices for non-exercising conditions. This is in agreement with unchanged mRNA and protein expression of PGÇ-1α, regulators of mitochondrial biogenesis and their downstream effectors in insulin-resistant relatives (11). However, comparable baseline fATPase between REL and CON does not allow conclusions on differences in mitochondrial number/function, but might simply reflect normal basal metabolic rate in both groups. Although overall mitochondrial function can be reduced in REL (11), it was found to be normal in patients with T2DM at baseline (7, 8, 27, 28) and only reduced upon insulin stimulation (7, 8). Several groups have further shown dissociation between the level of markers for mitochondrial content and in vitro oxidative capacity. Only some (29, 30), but not others (6, 9) found differences in mitochondrial function adjusted for mitochondrial mass.

This study demonstrates that short-term exercise training (i) uniformly raises myocellular basal metabolic rate as assessed with fATPase independently of insulin sensitivity in healthy humans, (ii) does not affect mean fATPase in REL, but (iii) identifies subgroups of REL with different exercise responses. In some REL, these conditions unmask a variation to increase ATP production reflecting altered adaptation of basal metabolic rate to short-term exercise
training. In the face of normal fasting glucose and FFA, hereditary factors seem to play an important role, as this variation in response to exercise training was also seen in carriers of the A-allele of the \textit{NDUFB6} SNP, rs540467, which has been associated with impaired insulin action and T2DM risk (12).

Several mechanisms could explain the rise in fATPase in CON and in RESP. While long-term exercise training possibly stimulates fat oxidation (18, 19) and electron transport chain activity (31) through increased mitochondrial size/number (19, 32), it is unlikely that mitochondrial biogenesis contributed to the rise in fATPase after short duration of training. Of note, it is conceivable that even if mitochondrial capacity is impaired, putative regulators of oxidative phosphorylation could increase and maintain the balance between ATP demand and supply and thereby baseline fATPase. We detected a variation occurring despite normal basal fATPase in insulin sensitive relatives. In NRES, the decreased fATPase affected adaptation of the myocellular basal metabolic rate and could therefore reflect reduced need or sufficient capability to supply ATP to meet energy demands or result from impaired oxidative ATP production (33).

This study also shows that IMCLs were not different from baseline at 48 hours after one week of three bouts of exercise. Interestingly, NRES had higher plasma FFA suggesting that increased lipid availability could contribute to impaired fATPase (5), although this might alternatively lead to increased mitochondrial function and biogenesis as demonstrated in rodent models (34, 35). Fat oxidation may rise regardless of insulin sensitivity even after one week of exercise training due to increased expression of enzymes involved in lipid metabolism (36). At 48 hours after one bout of exercise, insulin-stimulated glucose disposal (16, 26) and IMCL can be increased or unchanged (17, 37). Within 12 hours after moderate-to-high intensity exercise, whole body oxygen consumption returns to pre-exercise levels (38, 39) whereas both IMCL re-synthesis and lipid oxidation continued to rise up to more than 40 hours (38, 39). The post-exercise rise in fATPase would thereby result from increased ATP demand for augmented lipid turnover. Of note, a portion of the Pi/ATP exchange may be catalyzed by glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase. Although the contribution of anaerobic glycolysis to muscular ATP production is relevant during initial states of exercise and short-time high-intensity exercise, it ceases in the absence of muscle contraction and during aerobic resting conditions (40, 41). Of note, despite the known negative relationship between HCL and baseline insulin sensitivity at baseline (14), the insulin sensitivity improved after exercise training despite no reductions in IMCL and HCL. This is an important finding of the study pointing to independent regulation of insulin sensitivity and ectopic lipids by short term exercise training.

The absent fATPase response in NRES was recorded at 48 h after the last bout of exercise, when insulin secretion, G6P, pH and IMCL were not altered in all groups. Thus, acute effects occurring within the first 24 h after exercise such as stimulation of intramyocellular glucose transport/phosphorylation (42) and IMCL depletion (26, 37) unlikely contributed to fATPase alterations. Of note, exercise training slightly but significantly stimulated insulin sensitivity in both groups in agreement with previous studied T2DM relatives (16). Insulin resistant relatives respond to 6-weeks endurance training with increased insulin sensitivity and substrate oxidation (10, 16). On the other hand, combined diet and training for 2 weeks was required to improve insulin sensitivity and IMCL in T2DM (43). However, in the present study, insulin sensitivity failed to explain the fATPase
response suggesting that effects of short-term exercise training on mitochondrial function and insulin sensitivity can be dissociated in skeletal muscle. Although one cannot exclude that mitochondrial oxidation rises before changes in insulin sensitivity in healthy humans, previous studies measuring muscular metabolites and enzyme activities reported that lactate, phosphocreatine hydrolysis and glycogen depletion are reduced after 5 days of training whereas maximal succinate dehydrogenase activity as a surrogate of mitochondrial function increased only after 31 days (44). As insulin action was impaired in endurance trained individuals who stop exercise training, despite unchanged oxidative capacity (45), other mechanisms than oxidative capacity such as muscle glycogen synthesis likely contribute to changes in OGIS. At 48 hours of one bout of exercising, overweight insulin resistant relatives improved their insulin-stimulated glucose disposal associated with increased glycogen synthesis (16). This suggests that increased glycogen storage mainly accounts for improved insulin action after exercise (46). As our patients were not on hypercaloric diet, their glycogen stores were unlikely replenished to pre-exercise levels which is known to be an important modulator of insulin sensitivity for at least 72 h after the last exercise session (46).

The A-allele carriers of the NDUFB6 SNP, rs540467, showed a variation in the response to exercise in line with an inherited difference. NDUFB6 is among the genes encoding the respiratory chain, which show lower muscular expression in T2DM patients than controls (47). This SNP was also associated with resistance to an increase in OGIS after exercise training in line with a reported relationship with glucose disposal and risk of T2DM (12). The present study further supports the important role for this gene in ATP production in humans in vivo, but cannot determine whether these adaptations were related to changes in mitochondrial number, localization or function. In this context it is of note that activation of PPARs might improve insulin sensitivity by transcriptional control of mitochondrial function due to enhanced fatty acid oxidation and regulation of aerobic capacity. The Botnia study identified a common variant in the PPARγ gene (P12A) as one of the best predictors of future development of T2DM (48). Recent data provide evidence that even within 300 min after one bout of exercise expression and activation of PGC-1α, AMP-dependent protein kinase phosphorylation, nuclear respiratory factor-1 and cytochrome c oxidase are increased in lean but not in insulin-resistant obese humans (49). Although, previous studies suggest roles for PPARGC1A in oxidative phosphorylation, insulin action, VO2max and exercise response (12, 13, 47, 49), the present study did not find such associations for the PPARGC1A Gly482Ser gene variant probably due to lack of statistical power. Also, longer duration of training might be needed to reveal effects of this gene variant, as PPARGC1A Gly482Ser predicts endurance capacity (50).

The present study has some limitations. First, quantitative differences from previous studies might be due to the exercise protocols, degree of insulin resistance or the individual exercise capacity. To control for variations in exercise capacity, participants were exercising at 90% of their RCP, the highest work load at which oxidative phosphorylation is adequate for energy demand (51). Second, minor changes in amount and timing of caloric intake relative to exercise could have modulated OGIS and fATPase (52). But unchanged caloric intake and identical timing of meals render this possibility unlikely. Third, glucose ingestion during the OGTT before MRS measurements could have differently affected fATPase. However, the absence of differences in
plasma glucose between groups at 150 min of the OGTT and the start of MRS after further two hours and fATPase does not support such effect. Fourth, fATPase could have been affected by alterations of ATP concentrations which can increase up to 18 h after exercise (53). Such rise of ATP would have led to lower fATPase values thereby underestimating the actual ATP synthase flux. However, the observation of unchanged ATP/PDE ratios and the timing of measurement at 48 h after the last bout of exercise do not support alterations of ATP levels. Moreover, exchange rates, k1, which do not depend on substrate concentrations behaved similarly as fATPase between and within groups before and after training underlining that the observed differences reflect variation in ATP synthase flux. Finally, no muscle biopsies were taken so that neither mass-adjusted mitochondrial function nor possible changes in the expression of transcriptional modulators could be assessed. Unchanged plasma RBP-4 and visfatin do not exclude altered expression/activity of these factors.

In conclusion, training-induced increases of insulin sensitivity and ATP synthesis indicate an important role of early adaptation of basal metabolic rate in healthy humans. Conversely, some REL do not stimulate ATP production in response to short-term exercise training, nor do they have improved insulin sensitivity, and these same individuals carry a risk polymorphism in the NDUFB6 gene.

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REFERENCES


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**Table 1.** Baseline clinical and laboratory characteristics (means±SD, [95% CI]) of controls (CON) and first-degree relatives of type 2 diabetic patients (REL) and REL subgroups: responders (RESP) and non-responders (NRES) of ATP-synthesis to exercise training.

<table>
<thead>
<tr>
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<th>CON</th>
<th>REL</th>
<th>RESP</th>
<th>NRES</th>
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</thead>
<tbody>
<tr>
<td>N (female/male)</td>
<td>12 (6/6)</td>
<td>24 (13/11)</td>
<td>10 (4/6)</td>
<td>14 (9/5)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.83±0.08, [0.78-0.88]</td>
<td>0.85±0.05, [0.83-0.87]</td>
<td>0.87±0.04, [0.84-0.90]</td>
<td>0.84±0.05, [0.81-0.87]</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124±19, [112-135]</td>
<td>126±11, [121-130]</td>
<td>127±11, [119-135]</td>
<td>125±12, [118-132]</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82±10, [76-88]</td>
<td>83±8, [79-86]</td>
<td>82±8, [76-88]</td>
<td>84±8, [79-88]</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>5.3±0.3, [5.1-5.5]</td>
<td>5.4±0.3, [5.3-5.6]</td>
<td>5.5±0.4, [5.2-5.7]</td>
<td>5.4±0.3, [5.3-5.6]</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.87±0.50, [0.53-1.20]</td>
<td>1.23±0.70, [0.94-1.53]</td>
<td>1.32±0.57, [0.91-1.73]</td>
<td>1.18±0.80, [0.72-1.64]</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.7±0.45, [1.4-2.0]</td>
<td>1.5±0.4, [1.3-1.6]</td>
<td>1.4±0.4, [1.1-1.7]</td>
<td>1.5±0.4, [1.3-1.7]</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.0±0.7, [2.5-3.4]</td>
<td>3.5±0.9, [3.1-3.9]</td>
<td>3.8±0.9, [3.2-4.4]</td>
<td>3.3±1.0, [2.8-3.9]</td>
</tr>
<tr>
<td>Physical activity (scale 1 to 5)</td>
<td>2.7±0.3, [2.5-2.9]</td>
<td>2.9±0.3, [2.7-3.0]</td>
<td>2.9±0.3, [2.6-3.1]</td>
<td>2.9±0.3, [2.7-3.1]</td>
</tr>
<tr>
<td>VO₂max (ml.kg⁻¹.min⁻¹)</td>
<td>33.6±6.0, [29.8-37.5]</td>
<td>30.6±6.4, [27.8-33.3]</td>
<td>29.6±5.5, [25.7-33.6]</td>
<td>31.2±7.1, [27.1-35.3]</td>
</tr>
<tr>
<td>VO₂RCP (ml.kg⁻¹.min⁻¹)</td>
<td>26.2±4.8, [23.1-29.2]</td>
<td>22.8±5.7, [20.4-25.2]</td>
<td>22.1±4.7, [18.7-25.4]</td>
<td>23.3±6.4, [19.6-27.0]</td>
</tr>
</tbody>
</table>
### Table 2. Nutrient intake obtained from 24-hours dietary recalls (means±SD, [95% CI]) in controls (CON) and first-degree relatives of type 2 diabetic patients (REL). Dietary data after training were not available from one relative who lost the dietary record. There were no statistical differences within and between groups.

<table>
<thead>
<tr>
<th></th>
<th>CON Before</th>
<th>CON After training</th>
<th>REL Before</th>
<th>REL After training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (%)</td>
<td>40±10, [34-47]</td>
<td>37±8, [32-42]</td>
<td>37±10, [33-41]</td>
<td>35±8, [31-38]</td>
</tr>
<tr>
<td>n-3 fatty acids (%)</td>
<td>1±0, [1-2]</td>
<td>1±1, [1-2]</td>
<td>1±1, [1-1]</td>
<td>1±0, [1-1]</td>
</tr>
<tr>
<td>Cholesterol (g/day)</td>
<td>0.38±0.17, [0.27-0.50]</td>
<td>0.38±0.20, [0.26-0.51]</td>
<td>0.34±0.22, [0.25-0.44]</td>
<td>0.30±0.17, [0.22-0.37]</td>
</tr>
</tbody>
</table>

### Table 3. First-degree relatives of type 2 diabetic patients (REL) and of responder (RESP) and non-responder (NRES) of ATP synthesis to exercise training.*P<0.05 before vs. after exercise, ** P<0.05 CON vs. REL at baseline, $P<0.05$ CON vs. REL after exercise training.

<table>
<thead>
<tr>
<th></th>
<th>Before exercise</th>
<th>After Exercise</th>
<th>Before exercise</th>
<th>After Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>CON 4.9±0.3, [4.7-5.1]</td>
<td>4.9±0.3, [4.7-5.1]</td>
<td>RESP 5.0±0.4, [4.8-5.3]</td>
<td>5.0±0.4, [4.7-5.3]</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>CON 1.1±0.3, [0.9-1.3]</td>
<td>1.0±0.2*, [0.8-1.1]</td>
<td>RESP 1.3±0.5, [1.0-1.7]</td>
<td>1.2±0.3, [0.9-1.4]</td>
</tr>
<tr>
<td>C-peptide (nmol/l)</td>
<td>CON 1.6±0.4, [1.3-1.8]</td>
<td>1.5±0.4, [1.3-1.8]</td>
<td>RESP 2.4±0.8, [1.9-3.0]</td>
<td>2.3±1.0, [1.6-3.0]</td>
</tr>
<tr>
<td>QUICKI</td>
<td>CON 0.51±0.05, [0.48-0.54]</td>
<td>0.52±0.04, [0.50-0.54]</td>
<td>RESP 0.48±0.06, [0.44-0.52]</td>
<td>0.48±0.05, [0.44-0.51]</td>
</tr>
<tr>
<td>Insulinogenic index</td>
<td>CON 4.3±1.5, [3.3-5.2]</td>
<td>6.1±4.2, [3.4-8.8]</td>
<td>RESP 4.3±2.7, [2.4-6.2]</td>
<td>5.0±3.6, [2.4-7.5]</td>
</tr>
</tbody>
</table>
Table 4. Association between SNPs in the *NDUFB6* (rs540467) and *PPARGC1A* (rs8192678, Gly482Ser) genes and response to exercise training regarding stimulation of ATP synthesis (fATPase) and dynamic insulin sensitivity (OGIS) in responders and non-responders. Chi-square tests were performed to analyze associations between genotypes and response to exercise for fATPase and OGIS.

<table>
<thead>
<tr>
<th><em>NDUFB6</em> rs540467</th>
<th>G/G</th>
<th>G/A</th>
<th>A/A</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td><strong>fATPase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>14</td>
<td>4</td>
<td>1</td>
<td>0.02*</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>5</td>
<td>9</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>OGIS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>16</td>
<td>7</td>
<td>1</td>
<td>0.05*</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>3</td>
<td>6</td>
<td>1</td>
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</table>

<table>
<thead>
<tr>
<th><em>PPARGC1A</em> rs8192678</th>
<th>Gly/Gly</th>
<th>Gly/Ser</th>
<th>Ser/Ser</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td><strong>fATPase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>9</td>
<td>10</td>
<td>0</td>
<td>0.24</td>
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<tr>
<td>Nonresponders</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>OGIS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>11</td>
<td>11</td>
<td>2</td>
<td>0.64</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* p value indicating significance for a dominant model.
Figure Legends

**Figure 1.** $^{31}$P magnetic resonance spectrum from acquired at 3 Tesla employing a surface coil (TR = 15 s, NS = 16) positioned under the calf muscle of one participant. The spectrum shows intramyocellular phosphomonoesters (PME) including glucose-6-phosphate (G6P), inorganic phosphate (Pi), phosphodiesters (PDE), phosphocreatine (PCr) and adenosine-triphosphate (ATP). **Insert:** $^{31}$P spectra with saturation of $\gamma$-ATP (bottom) and with saturation mirrored around Pi (top), which was always used to account and correct for direct saturation of the resonance frequency pulse.

**Figure 2.** Dynamic insulin sensitivity as assessed from the oral glucose tolerance test (OGIS) in individuals without (CON, n=12) or with (REL, n=24) first-degree relatives with type 2 diabetes and in REL-subgroups responding (RESP, n=10) or not responding (NRES, n=14) with increased ATP-synthesis after exercise training sessions. Black horizontal bars indicate mean values of the respective groups.

* $p=0.049$ CON before vs. after, ** $p=0.012$ REL before vs. after, † $p=0.009$ RESP before vs. after, ‡ $p=0.012$ CON vs. REL before, § $p=0.003$, CON vs. RESP before, $^\ddagger$ $p=0.031$ CON vs. RESP after exercise

**Figure 3.** Flux through skeletal muscle ATP synthase (fATPase) in individuals without (CON, n=12) or with (REL, n=24) first-degree relatives with type 2 diabetes and in REL-subgroups responding (RESP, n=10) or not responding (NRES, n=14) with increased ATP-synthesis after exercise training sessions. Black horizontal bars indicate mean values of the respective groups.

* $p<0.001$ CON and NRES before vs. after, ** $p=0.002$, CON vs. REL after, † $p=0.010$ CON vs. RESP before, $^\ddagger$ $p=0.009$ RESP before vs. after, § $p=0.024$ RESP vs. NRES before, $^\ddagger$ $p<0.001$ CON vs. NRES after exercise

**Figure 4.** Absolute changes (delta; means±SEM) in dynamic insulin sensitivity (OGIS), flux through ATP synthase (fATPase) and lipid concentrations in liver (HCL) and soleus muscle (IMCL) in individuals without (CON, n=12) or with (REL, n=24) first-degree relatives with type 2 diabetes and in REL subgroups responding (RESP, n=10) or not responding (NRES, n=14) with increased ATP synthesis after exercise training sessions.

* $p=0.005$ CON vs. REL, ** $p<0.001$ RESP vs. NRES, $^\ddagger$ $p<0.001$ CON vs. NRES, † $p=0.014$ REL vs. RESP, $^\ddagger$ $p=0.024$ REL vs. NRES
Exercise training and ATP production

Figure 1.

Figure 2.

Dynamic insulin sensitivity (OGIS)
Figure 3.

ATP synthetic flux (fATPase)

Figure 4.

OGIS

fATPase

HCL

IMCL