Acquired obesity and poor physical fitness impair expression of genes of mitochondrial oxidative phosphorylation in monozygotic twins discordant for obesity

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Mustelin L, Pietiläinen KH, Rissanen A, Sovijärvi AR, Piirilä P, Naukkarinen J, Peltonen L, Kaprio J, Yki-Järvinen H. Acquired obesity and poor physical fitness impair expression of genes of mitochondrial oxidative phosphorylation in monozygotic twins discordant for obesity, Am J Physiol Endocrinol Metab 295: E148–E154, 2008. First published May 6, 2008; doi:10.1152/ajpendo.00580.2007.—Defects in expression of genes of oxidative phosphorylation in mitochondria have been suggested to be a key pathophysiological feature in familial insulin resistance. We examined whether such defects can arise from lifestyle-related factors alone. Fourteen obesity-discordant (BMI difference 5.2 ± 1.8 kg/m²) and 10 concordant (1.0 ± 0.7 kg/m²) monozygotic (MZ) twin pairs aged 24–27 yr were identified among 658 MZ pairs in the population-based FinnTwin16 study. Whole body insulin sensitivity was measured using the euglycemic hyperinsulinemic clamp technique. Transcript profiles of mitochondrial genes were compared using microarray data of fat biopsies from discordant twins. Body composition of twins was determined using DEXA and maximal oxygen uptake (V̇O₂max) and working capacity (Wmax) using a bicycle ergometer exercise test with gas exchange analysis. The obese cotwins had lower insulin sensitivity than their nonobese counterparts (M value 6.1 ± 2.0 vs. 9.2 ± 3.2 mg·kg⁻¹·LBM⁻¹·min⁻¹, P < 0.01). Transcript levels of genes involved in the oxidative phosphorylation pathway (GO:0006119) in adipose tissue were lower (P < 0.05) in the obese compared with the nonobese cotwins. The obese cotwins were also less fit, as measured by V̇O₂max (50.6 ± 6.5 vs. 54.2 ± 6.4 ml·kg⁻¹·LBM⁻¹·min⁻¹, for obese vs. nonobese, P < 0.05), Wmax (3.9 ± 0.5 vs. 4.4 ± 0.7 W/kg LBM, P < 0.01) and also less active, by the Baecke leisure time physical activity index (2.8 ± 0.5 vs. 3.3 ± 0.6, P < 0.01). This implies that acquired poor physical fitness is associated with defective expression of the oxidative pathway components in adipose tissue mitochondria.

Body composition; cardiorespiratory fitness; spiroergometry; gene expression

Obesity is associated with poor physical fitness (15) and insulin resistance (31, 32). Recent studies have suggested that genetic factors account for a substantial fraction (estimates of 50–90%) of the population variance in BMI (39, 65), insulin action (23–52%) (16, 27, 35, 46, 60) and physical activity (32–79%) (24, 41, 70). These results raise the possibility that shared genetic influences underlie the association between obesity, insulin resistance, and fitness. Recently, it has been suggested that mitochondrial dysfunction might be a key factor in development of insulin resistance (37). Several studies have reported type 2 diabetic patients and “prediabetic” insulin-resistant subjects to have impaired structure and function of mitochondria (28, 48, 53, 54, 66) and decreased expression of genes encoding key enzymes in oxidative metabolism and mitochondrial function (47, 52). Petersen et al. (54) reported reduced mitochondrial phosphorylation in insulin-resistant offspring of patients with type 2 diabetes compared with insulin-sensitive control subjects. Decreased oxidative capacity of mitochondria in insulin-resistant subjects was therefore suggested to be due to an inherited genetic defect (54).

Studies of monozygotic (MZ) twins have greatly contributed to our understanding of environmental and lifestyle factors impacting human phenotypes (43). Among genetically identical individuals, intrapair differences are caused ultimately by environmental factors and can thus be used to study relationships while controlling for genetic influences. In the present study, we studied genetically identical twins who were either concordant or discordant for obesity to determine whether lifestyle-related factors (obesity and/or physical inactivity) decrease V̇O₂max and influence the transcript levels of genes involved in mitochondrial oxidative phosphorylation.

MATERIALS AND METHODS

Subjects. The study participants were recruited from the FinnTwin16 cohort (26, 62), a population-based, longitudinal study of five consecutive birth cohorts (1975–1979) of twins and their siblings and parents.

Twin pairs to the current study were recruited on the basis of their responses to questions on weight and height at the age of 23–27 yr. After all MZ twin pairs (n = 658) had been screened, we identified 18 pairs with a reported BMI difference of at least 4 kg/m², such that one cotwin was nonobese (mean BMI 25 kg/m²), whereas the other was obese (mean BMI 30 kg/m²) (17, 25, 58, 59). Fourteen of these pairs

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(8 male and 6 female pairs) participated. In addition to these discordant pairs, we studied 10 concordant MZ pairs (5 male and 5 female pairs) with a BMI difference of less than 2 kg/m².

The subjects were healthy and normotensive and did not use any medications except for oral contraceptives. Their weight had been stable for at least 3 mo prior to the study. Two pairs had a family history of type 2 diabetes. Monozogosity was confirmed by genotyping of 10 informative genetic markers (59). The subjects provided written informed consent. The protocol was designed and performed according to the principles of the Helsinki Declaration and was approved by the Ethics Committee of the Helsinki University Central Hospital.

**Study design.** In each subject, whole body insulin sensitivity, body composition, and cardiorespiratory fitness were measured. The measurement of whole body insulin sensitivity was performed using the euglycemic insulin clamp technique after an overnight fast. Prior to the clamp, a needle biopsy of abdominal subcutaneous adipose tissue was taken from 13 discordant pairs, as previously described (57). The sample used for microarray analysis was immediately frozen in liquid nitrogen and stored at −80°C until isolation of RNA.

**Whole body insulin sensitivity.** Whole body insulin sensitivity was measured using the euglycemic hyperinsulinemic clamp technique as previously described (10, 58). The rate of the continuous insulin infusion was 1 μU·kg⁻¹·min⁻¹.

**Gene expression in adipose tissue.** Total RNA was prepared from frozen fat tissue (on average 250 mg) using the RNeasy Lipid Tissue Mini Kit (Qiagen) according to the manufacturer’s protocol. Quality of RNA was analyzed using the 2100 Bioanalyzer platform (Agilent Technologies). Two micrograms of total RNA was treated according to the conventional Affymetrix eukaryotic RNA labeling protocols (Affymetrix, Santa Clara, CA). Fifteen micrograms of biotin-labeled cRNA was fragmented according Affymetrix eukaryotic sample protocol. Hybridization, staining, and washing of the Affymetrix U133 Plus 2.0 chips were performed using the Affymetrix Fluidics Station 450 and Hybridization Oven 640 under standard conditions. Preprocessing of expression data was done using the GC-RMA algorithm. Prior to analysis, the expression values were cotwin normalized, which involved dividing the obese twin’s expression values with those of the thin cotwin to correct for the identical genetic background.

The pathway analysis was done with an in-house nonparametric analysis algorithm, where the objective is to find the optimal regulated pathway compositions without prior criteria for significance for individual genes’ significant regulation or arbitrary P value/fold change cutoffs, as described previously (57). Briefly, the probe sets were ranked by the median fold change values between obese and lean twins to “upregulated” and “downregulated” vectors. The Affymetrix ProbeSetIDs were mapped to human genes using cross-references in the Ensembl database, and the genes were queried for their GO annotations. The topology of the GO tree (DAG tree) was fully utilized by enumerating all available routes toward the root of the GO tree and adding all encountered vertexes as GO annotations of the given gene. For detecting the affected GO gene groups (“pathways”), an iterative hypergeometric distribution P value-based calculation was used, where the objective is to find the optimum P value for a set of genes that belong to the same annotated GO gene collection (maximal regulation for the pathway). Furthermore, the data were exhaustively permuted by randomizing the gene ranks for each GO category, and an empirical P value was interpreted from the distribution of 10,000 permutation cycles. The oxidative phosphorylation pathway (GO: 0006119) was queried as part of these pathway analyses (57) of the genome-wide microarray expression profiles. The global pathway analyses evaluate the significance of all individual GO categories while taking into account the structure of the tree.

Gene transcription was compared using previously reported real-time PCR data (25, 56) and current Affymetrix data. The relationships were as follows: 11β-hydroxysteroid dehydrogenase-1 (11β-HSD-1; r = 0.68, P = 0.005), glucocorticoid receptor-α (r = 0.45, P = 0.11), adiponectin [r = 0.06, not significant (NS)], lipoprotein lipase (LPL; r = 0.63, P = 0.012), peroxisome proliferator-activated receptor-γ (PPARY; r = 0.77, P = 0.0001), leptin (r = 0.52, P = 0.01), CD68 (r = 0.09, NS), IL-6 (r = −0.26, NS), and TNF-α (r = −0.05, NS).

**Body composition measurements.** Body composition, including fat mass, lean body mass (LBM), and percent whole body fat was measured by dual-energy X-ray absorptiometry (DEXA; Lunar Prodigy, Madison, WI; software version 2.15) (44).

**Cardiorespiratory exercise test.** Work-conducted maximal exercise test with gas exchange analysis (spiroergometry) was performed using an electrically braked bicycle ergometer (900 ERG Ergometer; Marquette Hellige, Marquette Medical Systems) and by using a breathing-by-breath gas exchange analysis system (Vmax 229, Sensormedics) with the test subject sitting upright. The initial workload was 40 W for women and 50 W for men. It was then increased by 40 and 50 W, respectively, at 3-min intervals until exhaustion [19–20/20 on the Borg scale for perceived exertion or respiratory quotient (RQ = VO₂/VO₂) of at least 1.1]. Blood pressure was measured manually using a stethoscope and a sphygmomanometer (Erka) from the left arm before, during, and 6–10 min after exercise. A 12-lead ECG (Mason-Likar) was continuously monitored and recorded during the exercise test using a computerized device (Case12, Marquette). For measurement of respiratory gases, a tightly attached face mask (Rudolph series 7910, Hans Rudolph) with a dead space of 185 ml was used. Arterial O₂ saturation (SaO₂) was assessed noninvasively with two pulse oximeters (Datex-Ohmeda 3900 and Datex-Ohmeda 3800; Datex-Ohmeda). One sensor was attached to the earlobe and one to the left middle finger.

A mass flow sensor of the gas exchange device (Sensormedics) was used to measure forced expiratory volume in one second before exercise, and tidal volume and minute ventilation during exercise. From the breath-by-breath recordings ventilatory and gas exchange variables were averaged at 30-s intervals using microcomputer-assisted equipment. The variables used for analysis are explained below.

Maximal oxygen uptake (VO₂max) was defined as the peak oxygen uptake. Maximal working capacity (Wmax) was defined as the mean workload during the last 3 min of exercise. Mechanical efficiency (73) and anaerobic threshold (AT) assessed from the slope change of VO₂/VO₂ were also calculated.

**Physical activity.** Physical activity was assessed using the Baecke questionnaire (2). Work index refers to physical activity at work, sport index to sport during leisure time, and leisure time index to physical activity during leisure time excluding sports.

**Statistical analyses.** Student’s paired t-test was used to compare the means between the leaner and heavier cotwins for normally distributed variables and Wilcoxon’s signed rank test for the intrapair comparison of nonnormally distributed data. Statistical analyses were performed using Stata 8.2 for Windows software (StataCorp, College Station, TX). Calculation of correlation coefficients between acquired intrapair differences in body composition and intrapair differences in fitness or metabolic measurement controls for genetic and shared environmental influences within MZ pairs. Pearson correlation and linear regression was used in the intrapair and individual univariate analyses. Multiple linear regression models were used to control for confounding factors in some analyses. When using individual twins, the P values were corrected for clustered sampling of cotwins within pairs by survey methods (61). To combine male and female pairs, we used sex-specific z-transformed values (i.e., mean of 0 and SD of 1) of the study variables in all analyses.

**RESULTS**

**Body composition.** A pairwise comparison of the critical traits for obesity-discordant and -concordant pairs is shown in Table 1. In the discordant pairs, the heavier cotwin had, on average, a 5.4 kg/m² higher BMI, a 9.4% higher fat percentage, 12.9 kg more fat, and 2.4 kg more LBM than the leaner cotwin.
Table 1. Characteristics of MZ cotwins discordant and concordant for obesity

<table>
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<tr>
<th></th>
<th>Discordant Pairs</th>
<th>Concordant Pairs</th>
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<tbody>
<tr>
<td></td>
<td>Heavier</td>
<td>Leaner</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Age, yr</td>
<td>25.7±1.5</td>
<td>25.6±1.5</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>2588±481</td>
<td>2464±526</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171±9</td>
<td>171±9</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>89.8±9.4</td>
<td>74.5±8.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.6±2.0</td>
<td>25.4±1.8</td>
</tr>
<tr>
<td>%Body fat</td>
<td>38.3±6.5</td>
<td>29.5±8.5</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>34.0±5.1</td>
<td>21.4±5.7</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>52.8±9.8</td>
<td>50.4±10.2*</td>
</tr>
<tr>
<td>M value/LBM, mg/kg</td>
<td>6.1±2.1</td>
<td>9.1±2.9†</td>
</tr>
<tr>
<td>Blood hemoglobin, g/l</td>
<td>144±11</td>
<td>140±11</td>
</tr>
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</table>

Data shown as mean ± SD. MZ, monozygotic; LBM, lean body mass. *P < 0.05, †P < 0.01, ‡P < 0.001 (Student’s paired t-test).

In the discordant pairs, the respective differences were 1.0 kg/m², 2.2%, 2.1 kg fat, and 0.3 kg LBM.

Insulin sensitivity. The obese cotwins had significantly lower whole body insulin sensitivity (M value) than their nonobese counterparts (Table 1). Intrapair differences in whole body insulin sensitivity were associated with those in VO2max (P = 0.05, †P < 0.01, ‡P < 0.001) or VCO2max. The oxidative phosphorylation pathway was found to significantly correlate with VO2max. The oxidative phosphorylation pathway was identified in the obese compared with the nonobese cotwins (nominal P < 0.001, permuted P < 0.05). The rank of the oxidative phosphorylation pathway was 123 among 184 significantly downregulated pathways. Among 68 genes in the pathway, 31 were responsible for the optimum pathway composition (Fig. 1). The mean centroid [describing the average activity of the regulated part of the pathway, as described by Mootha et al. (47)] was calculated for the oxidative phosphorylation pathway and used to examine possible correlation with VO2max.

DISCUSSION

The present study shows that, independent of genetic factors, obesity is associated with poor fitness, low insulin sensitivity, and decreased transcript levels of genes involved in mitochondrial oxidative phosphorylation. This conclusion is based on a study of a group of genetically identical MZ twin pairs discordant or concordant for BMI. MZ twin pairs discordant for obesity offer a unique opportunity to study effects of acquired obesity independently of confounding genetic influences while matching for age, sex, and many socioeconomic, childhood, and familial factors. Monozygosity in the present study was confirmed by genotyping of 10 informative genetic markers (59). This study design controls in an ideal way for the genetic effect on obesity. Obese cotwins were shown to have lower whole body insulin sensitivity, VO2max, and Wmax relative to lower mechanical efficiency than the nonobese one (P < 0.05). Regarding physical activity, the obese cotwin had a lower leisure time index than the nonobese one (2.8 vs. 3.3, obese vs. lean, P < 0.01). Work or sport indexes did not differ between the cotwins (2.4 vs. 2.4 and 3.1 vs. 3.0, respectively). Correlations between VO2max/LBM and the physical and biochemical characteristics are shown in Table 3.

Transcript levels. The genome-wide transcript profiling of fat biopsies showed that genes involved in the mitochondrial oxidative phosphorylation pathway were significantly downregulated in the obese compared with the nonobese cotwins (nominal P < 0.001, permuted P < 0.05). The rank of the oxidative phosphorylation pathway was ranked in 184 significantly downregulated pathways. Among 68 genes in the pathway, 31 were responsible for the optimum pathway composition (Fig. 1). The mean centroid [describing the average activity of the regulated part of the pathway, as described by Mootha et al. (47)] was calculated for the oxidative phosphorylation pathway and used to examine possible correlation with VO2max. The oxidative phosphorylation pathway was identified in the obese compared with the nonobese cotwins (nominal P < 0.001, permuted P < 0.05). The rank of the oxidative phosphorylation pathway was ranked in 184 significantly downregulated pathways. Among 68 genes in the pathway, 31 were responsible for the optimum pathway composition (Fig. 1). The mean centroid [describing the average activity of the regulated part of the pathway, as described by Mootha et al. (47)] was calculated for the oxidative phosphorylation pathway and used to examine possible correlation with VO2max.

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<td>Heavier</td>
<td>Leaner</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Wmax, W</td>
<td>197±41</td>
<td>215±56</td>
</tr>
<tr>
<td>Wmax/LBM, W/kg LBM</td>
<td>3.9±0.5</td>
<td>4.4±0.6†</td>
</tr>
<tr>
<td>VO2max, l/min</td>
<td>2.60±0.59</td>
<td>2.67±0.70</td>
</tr>
<tr>
<td>VO2max/BW, ml·kg·BW⁻¹·min⁻¹</td>
<td>29.2±4.7</td>
<td>36.0±7.4‡</td>
</tr>
<tr>
<td>VO2max/LBM, ml·kg·LBM⁻¹·min⁻¹</td>
<td>50.8±6.2</td>
<td>54.2±6.1*</td>
</tr>
<tr>
<td>Mechanical efficiency, %</td>
<td>22.0±1.4</td>
<td>23.1±1.3*</td>
</tr>
<tr>
<td>RQ</td>
<td>1.1±0.04</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>AT, l/min</td>
<td>1.3±0.5</td>
<td>1.5±0.5</td>
</tr>
<tr>
<td>AT/LBM, ml·kg·LBM⁻¹·min⁻¹</td>
<td>25.3±7.2</td>
<td>30.2±6.0*</td>
</tr>
<tr>
<td>Resting heart rate, min⁻¹</td>
<td>78±9</td>
<td>75±11</td>
</tr>
<tr>
<td>Maximal heart rate, min⁻¹</td>
<td>187±7</td>
<td>187±10</td>
</tr>
<tr>
<td>Resting systolic BP, mmHg</td>
<td>133±15</td>
<td>126±15*</td>
</tr>
<tr>
<td>Resting diastolic BP, mmHg</td>
<td>71±12</td>
<td>68±8</td>
</tr>
<tr>
<td>Maximal systolic BP, mmHg</td>
<td>193±30</td>
<td>190±24</td>
</tr>
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</table>

Data shown as mean ± SD. VO2max, maximal O2 uptake; Wmax, maximal working capacity; BW, total body weight; RQ, respiratory quotient (VCO2max/VO2max); AT, anaerobic threshold; BP, blood pressure. *P < 0.05, †P < 0.01, ‡P < 0.001 (Student’s paired t-test).
their LBM than their nonobese counterparts. These physiological changes were accompanied by the significantly lower transcript levels of genes involved in the mitochondrial oxidative phosphorylation pathway in adipose tissue.

\[ V\dot{O}_2\text{max} \]

was reduced in the obese compared with the nonobese twin when expressed per kilogram LBM and per kilogram body weight but not when expressed as milliliters per minute. The finding that \( V\dot{O}_2\text{max} \) per minute is not negatively influenced by obesity is consistent with several studies (9, 18, 36, 40, 42, 63), as is the finding that \( V\dot{O}_2\text{max} \) per kilogram total body weight is lower in obese than in nonobese subjects (9, 12, 18, 22, 36, 42, 63, 64). Since muscle tissue is the main site of \( V\dot{O}_2 \) during maximal exertion, \( V\dot{O}_2\text{max} \) divided by LBM seems the most unbiased way of comparing cardiorespiratory fitness of individuals of different sizes (11, 72).

Previous studies expressing \( V\dot{O}_2\text{max} \) per kilogram LBM have shown either decreased (9, 22, 23, 67, 69) or unchanged (12, 18, 34, 40) \( V\dot{O}_2\text{max} \) in obese compared with nonobese subjects. Possibly, genetic differences as well as those in age, sex, family environment, and physical activity could, together with small sample sizes, have confounded comparison of obese and nonobese subjects in the latter studies (12, 18, 34, 40).

Several studies have reported impaired mitochondrial activity (28, 48, 53–55, 66) or decreased transcript levels of genes involved in mitochondrial oxidative metabolism (47, 52) in skeletal muscle of insulin-resistant subjects. Kelley et al. (28) reported smaller mitochondria and reduced activity of the electron transport chain in obese subjects and type 2 diabetic patients compared with normal-weight, insulin-sensitive control subjects. Patti et al. (52) and Mootha et al. (47) both found reduced expression of genes encoding proteins involved in mitochondrial oxidative metabolism in skeletal muscle from insulin-resistant subjects. Reduced rates of mitochondrial phosphorylation (54), impaired insulin-stimulated ATP synthesis (55), reduced mitochondrial density (48), and prolonged phosphocreatine recovery half-time (66) have also been found to characterize skeletal muscle of type 2 diabetic patients.

Table 3. Correlations between \( V\dot{O}_2\text{max}/\text{kg LBM} \) and physical and biochemical characteristics in all MZ twins (n = 48)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Correlation Coefficient</th>
</tr>
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<tbody>
<tr>
<td>Birth weight, g</td>
<td>-0.15</td>
</tr>
<tr>
<td>Height, cm</td>
<td>-0.15</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>-0.44†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>-0.42†</td>
</tr>
<tr>
<td>%Body fat</td>
<td>0.16</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>0.22</td>
</tr>
<tr>
<td>M value/LBM, mg·kg·LBM⁻¹·min⁻¹</td>
<td>0.45†</td>
</tr>
<tr>
<td>Resting energy expenditure, kJ/day</td>
<td>-0.26</td>
</tr>
<tr>
<td>Mean adipose tissue gene expression of oxidative phosphorylation pathway (mean-centroid)</td>
<td>0.42*</td>
</tr>
</tbody>
</table>

* \( n = 22 \) (twins of discordant pairs for whom both gene expression data and \( V\dot{O}_2\text{max} \) were available). *\( p < 0.05 \), †\( p < 0.01 \) (Pearson correlation corrected for clustered twin data).
These results supported the concept that mitochondrial dysfunction and subsequent impairments in the oxidative metabolism characterize insulin-resistant subjects, possibly representing critical etiological factors (37). Very recently, however, Boushel et al. (7) suggested that mitochondrial function is normal in type 2 diabetes. They showed that, although overall muscle oxidative capacity was lower in the type 2 diabetic patients, their mitochondrial function was unimpaired. They thus concluded that the impairment of oxidative phosphorylation and electron transport can be attributed to diminished mitochondrial content in the diabetic muscle.

When pursuing the explanation for the discrepant findings in the above studies, physical activity/fitness of the study groups needs to be considered. In the study by Mootha et al. (47), subjects with impaired glucose tolerance as well as those with type 2 diabetes had significantly lower \( \text{V}^{\text{O}_2}_\text{max} \) than those with normal glucose tolerance. This could indicate a lower level of physical activity in the insulin-resistant study groups compared with the control group, although this was not specifically assessed. Schauren-Hinderling et al., on the other hand, found no significant difference in \( \text{V}^{\text{O}_2}_\text{max} \) between obese subjects with type 2 diabetes and similarly obese subjects with normal glucose tolerance (66). Since there was a trend toward better \( \text{V}^{\text{O}_2}_\text{max} \) in the non-insulin-resistant subjects, the nonsignificant result might simply reflect the small study sample (12 diabetic subjects and 9 nondiabetic control subjects). In only two of the above studies (54, 55) were the study groups matched for physical activity. The method of physical activity assessment was not, however, explained in the studies by Petersen et al. (54, 55). Additionally, two studies investigated only subjects defined as sedentary (48, 52) but presented no further data on their physical activity. In conclusion, none of the studies reporting mitochondrial dysfunction in insulin-resistant subjects properly measured physical activity and/or fitness, although exercise training is known to increase mitochondrial content and oxidative capacity of skeletal muscle fibers (1, 19–21) as well as to enhance insulin sensitivity (6, 8, 49, 50, 68) and prevent progression of impaired glucose tolerance to type 2 diabetes (13, 30, 33, 51).

In the present study, we found significant reduction of transcript levels of genes encoding components of mitochondrial oxidative phosphorylation in adipose tissue in obese monozygotic cotwins. We recognize the limitation of the use of adipose tissue instead of skeletal muscle tissue. However, our results of fat biopsies are in line with previous studies reporting reduced oxidative metabolism and downregulation of genes involved in oxidative phosphorylation in obese and insulin-resistant subjects’ muscle (28, 47, 48, 52–55, 66). The finding that this is also true in obese and nonobese subjects with identical genes is novel. It shows that lifestyle-related factors, mainly physical inactivity and acquired obesity, are enough to significantly impair expression of genes encoding proteins involved in mitochondrial oxidative phosphorylation. This is in agreement with the findings of Boushel et al. (7) as well as intervention studies reporting improvements of mitochondrial function in response to physical activity (45, 71).

Although we found that the reduced transcript levels of genes encoding mitochondrial oxidative phosphorylation in obesity is influenced by environmental and acquired factors, it does not exclude the possibility that genetic factors contribute to regulation of mitochondrial oxidative metabolism, as has been shown for \( \text{V}^{\text{O}_2}_\text{max} \) (4, 5, 14, 29, 38), physical activity (24, 41), and the \( \text{V}^{\text{O}_2}_\text{max} \) response to physical training (3). The heritability estimates for \( \text{V}^{\text{O}_2}_\text{max} \) vary between 50 and 67% in the two most recent studies (4, 38).

In conclusion, acquired obesity decreases physical fitness, whole body insulin sensitivity, and expression of mitochondrial genes involved in oxidative phosphorylation independently of genetic influences. These data suggest that physical inactivity may have contributed to the defects in mitochondrial oxidative phosphorylation described in type 2 diabetic patients and prediabetic subjects. The latter possibility, however, needs to be tested in an intervention study.

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REFERENCES

13. Eriksson J, Taimela S, Koivisto VA. Exercise and the metabolic syn-

14. Fagard R, Bielen E, Amery A. Heritability of aerobic power and
anaerobic energy generation during exercise. J Appl Physiol 70: 357–362,

15. Fogelholm M, Malmberg J, Suni J, Santtila M, Kyrolainen H, Man-
tysaari M. Waist circumference and BMI are independently associated
with the variation of cardio-respiratory and neuromuscular fitness in young

16. Freeman MS, Mansfield MW, Barrett JH, Grant PJ. Heritability of
features of the insulin resistance syndrome in a community-based study of

17. Gertow K, Pietilainen KH, Yki-Jarvinen H, Kaprio J, Rissanen A,
Fagard R, Bielen E, Amery A. Heritability of aerobic power and


19. He J, Watkins S, Kelley DE. Skeletal muscle lipid content and oxidative
enzyme activity in relation to muscle fiber type in type 2 diabetes and

1984.

21. Hood DA. Invited review: contractile activity-induced mitochondrial


23. Huttunen NP, Knip M, Pietilainen KH, Ahrlund-Richter L, Hamsten A,
Yki-Jarvinen H. Overexpression of 11beta-hydroxysteroid dehydrogenase-1 in adipose tissue is associated with acquired obesity and
features of insulin resistance: studies in young adult monozygotic twins.

24. Kaprio J, Pulkkinen L, Rose RJ. Genetic and environmental factors in
health-related behaviors: studies on Finnish twins and twin families. Twin

Eriksson J, Stengard J, Kesaniemi YA. Concordance for type 1 (insulin-
dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia 35: 1066–1067,

26. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria
in human skeletal muscle in type 2 diabetes. Diabetes 51: 2944–2950,
2002.

27. Klissouras V, Pirmay F, Petit JM. Adaptation to maximal effort: genetics

28. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin

29. Koltermann OG, Insel J, Saekow M, Olefsky J. Mechanisms of insulin
resistance in human obesity: evidence for receptor and postreceptor

2000.

31. Laaksonen DE, Lindstrom J, Lakka TA, Eriksson JG, Niskanen L,

32. Lehtovirta M, Kaprio J, Forsblom C, Eriksson J, Tuomilehto J, Groop L. Insulin sensitivity and insulin secretion in monogenic and


