HIGHLIGHTED TOPIC | Role of Exercise in Reducing the Risk of Diabetes and Obesity

PPARGC1A genotype (Gly482Ser) predicts exceptional endurance capacity in European men

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Lucia, Alejandro, Félix Gómez-Gallego, Inês Barroso, Manuel Rabadán, Fernando Bandrés, Alejandro F. San Juan, José L. Chicharro, Ulf Ekelund, Soren Brage, Conrad P. Earnest, Nicholas J. Wareham, and Paul W. Franks. PPARGC1A genotype (Gly482Ser) predicts exceptional endurance capacity in European men. J Appl Physiol 99: 344–348, 2005. First published February 10, 2005; doi:10.1152/japplphysiol.00037.2005.—Animal and human data indicate a role for the peroxisome proliferator-activated receptor-γ coactivator 1α (PPARGC1A) gene product in the development of maximal oxygen uptake (V\textsubscript{O\textsubscript{2 max}}), a determinant of endurance capacity, diabetes, and early death. We tested the hypothesis that the frequency of the minor Ser482 allele at the PPARGC1A locus is lower in World-class Spanish male endurance athletes (cases) [n=104; mean (SD) age: 26.8 (3.8) yr] than in unfit United Kingdom (UK) Caucasian male controls [n=100; mean (SD) age: 49.3 (8.1) yr]. In cases and controls, the Gly482Ser genotype met Hardy-Weinberg expectations (P>0.05 in both groups tested separately). Cases had significantly higher V\textsubscript{O\textsubscript{2 max}} [73.4 (5.7) vs. 29.4 ml•kg\textsuperscript{-1}•min\textsuperscript{-1} (3.8); P<0.0001] and were leaner [body mass index: 20.6 (1.5) vs. 27.6 kg/m\textsuperscript{2} (3.9); P<0.0001] than controls. In unadjusted χ\textsuperscript{2} analyses, the frequency of the minor Ser482 allele was significantly lower in cases than in controls (29.1 vs. 40.0%; P=0.01). To assess the possibility that genetic stratification could confound these observations, we also compared Gly482Ser genotype frequencies in Spanish (n=164) and UK Caucasian men (n=381) who were unselected for their level of fitness. In these analyses, Ser482 allele frequencies were very similar (36.9% in Spanish vs. 37.5% in UK Caucasians, P=0.83), suggesting that confounding by genetic stratification is unlikely to explain the association between Gly482Ser genotype and endurance capacity. In summary, our data indicate a role for the Gly482Ser genotype in determining aerobic fitness. This finding has relevance from the perspective of physical performance, but it may also be informative for the targeted prevention of diseases associated with low fitness such as Type 2 diabetes.

single nucleotide polymorphism; maximal oxygen uptake; metabolism; genetics; diabetes

A low maximal oxidative capacity, as defined by maximal oxygen uptake (V\textsubscript{O\textsubscript{2 max}}), independently predicts diabetes (22, 37) and early death (16–18, 38). V\textsubscript{O\textsubscript{2 max}} is determined by modifiable factors that include physical activity level (1), cigarette smoking (31), and micro- and macrovascular disease (14), and by unmodifiable factors such as genotype (6, 27) and the intrauterine environment (5). Several candidate genes have been identified that appear to influence V\textsubscript{O\textsubscript{2 max}} (27), of which one, PPARGC1A, is a particularly promising candidate, owing to the consistency of supportive data in rodents (3, 12, 19, 32, 33) and humans (23, 25, 28, 30, 34).

Several studies have reported association between a common variant in the PPARGC1A gene (Gly482Ser) and Type 2 diabetes, in which the frequency of the Ser482 allele is higher in diabetic patients than normal glucose-tolerant controls (9, 15, 24). More recently, healthier older Danish carriers of the Ser482 allele have also been reported to have lower levels of PPARGC1A and PPARGC1B mRNA by comparison with Gly482 allele homozygotes (20). The PPARGC1A gene is a coactivator of a subset of genes that control oxidative phosphorylation, which have been termed the OXPHOS genes. Control of oxidative phosphorylation by the OXPHOS genes is achieved through the regulation of mitochondrial biogenesis, glucose and lipid transport and oxidation (32, 34), and skeletal muscle fiber-type formation (19). V\textsubscript{O\textsubscript{2 max}} is positively correlated with OXPHOS mRNA levels in human skeletal muscle (23), and OXPHOS gene expression is coordinately downregulated in diabetic skeletal muscle (23).

In animals (3, 12, 32, 33) and humans (25, 26, 28, 30, 34), exercise training increases PPARGC1A mRNA levels, and transgenic overexpression of PPARGC1A mRNA corresponds with an increased resistance by contracting muscle to fatigue (19), indicating that PPARGC1A and exercise are part of a coregulatory feedback loop.

The use of extreme phenotype cohorts (i.e., groups of people who exhibit extreme levels of a given outcome trait) can help maximize statistical power in studies that seek to test hypotheses of genetic association. In the present study, we employed...
this approach to test the hypothesis that the minor Ser482 allele at the PPARGC1A locus is less frequent in world-class endurance athletes than in unfit controls.

METHODS

Selection of the Participants and Metabolic Tests

Cases. Written consent was obtained from each subject according to the guidelines of the local institutional ethics committee (Universidad Europea de Madrid, Spain), which approved this study. The study adhered to the principles of the Declaration of Helsinki.

The case group comprised 50 unrelated male World-class Spanish riders from the four best professional cycling teams who ranked among the top 65–70 Spanish cyclists in terms of a 3-wk stage race performance in this country. We specifically chose professional cyclists for this study who met the following criteria: each cyclist 1) had previously been enrolled on a professional cycling team, 2) had at least 2 yr experience in the professional category of the International Cycling Union, and 3) had participated in a classic 3-wk stage race from 1999 to 2004. During this period, 11 cyclists won at least one mass-start stage or time trial of the Tour de France, Giro d’Italia, or Vuelta a España, and 7 and 11 were among the top 3 and top 10 finishers, respectively, in these classic cycling races.

We recruited 54 male runners for this study who were the best World- and Olympic-class Spanish middle- and long-distance track runners, as determined by finishing positions during international competitions for the 1999–2004 period. Their training loads typically included more than 150 km/wk (150–200 km/wk) and approached 250 km/wk in marathoners during some periods of the year. Athletes within this group included individuals who had placed within the top three at World, European, and Olympic Championships during the years 1999–2004. V˙O2max was measured during an incremental load exercise stress test until exhaustion on a cycle-ergometer (cyclists) or treadmill (runners), as described in detail previously (21).

Spanish reference population. Written consent was obtained from each subject according to the guidelines of the local institutional ethics committee (Universidad Europea de Madrid, Spain), which approved this study. The study adhered to the principles of the Declaration of Helsinki. A group of 164 healthy unrelated Spanish men (aged 18–55 yr) served as the Spanish reference population for the ethnic comparison of Gly482Ser genotype frequencies. These individuals were anonymous blood donors in whom information was only available for age, ethnicity, genotype, and general health status and were thus unselected for their level of fitness.

United Kingdom reference population and controls. The United Kingdom (UK) reference and control groups were selected from the Medical Research Council (MRC) Ely Study, a continuing population-based cohort study in Ely, Cambridgeshire, the design of which has been described previously (10, 11). In brief, volunteers attended the clinic at 8:30 AM having fasted since 10 PM the previous evening. After an explanation of laboratory procedure, all participants provided written, informed consent. Height and weight were measured in light clothing, and blood was drawn through a cannulated artery. Ethical permission for the study was granted by the Cambridge Local Research Ethics Committee, which approved this study. The study adhered to the principles of the Declaration of Helsinki.

Anthropometric and genetic data were available in 381 men. This group was unselected for their level of cardiorespiratory fitness and constituted the sample for the comparison of allele frequencies between ethnic groups (i.e., the UK reference population).

Of the 381 participants in the UK reference population, V˙O2max was measured in a subset of 200 individuals. V˙O2max was assessed during an incremental load exercise stress test on a bicycle ergometer, as described previously in detail (10, 11). This sample was stratified above and below the group median for V˙O2max, to constitute the “fit” (n = 100) and “unfit” (n = 100) controls groups.

Genetic Analyses

DNA extraction and genotyping was undertaken using previously described methods (4). Genotyping was performed using an adaptation of the fluorescence polarization template-directed incorporation method described by Chen et al. (7). In short, primer extension preamplified DNA samples were polymerase chain reaction amplified in 8-μl reactions with primers flanking the variant site; unincorporated 2-deoxynucleotide 5′-triphosphate and remaining unused primer were degraded by exonuclease I and shrimp alkaline phosphatase at 37°C for 45 min before the enzymes were heat inactivated at 95°C for 15 min. At the end of the reaction, the samples were held at 4°C. Single base primer extension reactions were performed as previously described (7), and allele detection was performed by measuring fluorescence polarization on an LJL Analyst fluorescent reader (Molecular Devices, Sunnyvale, CA). The primer extension preamplified protocol was specifically developed and tested to ensure that allele bias was not introduced during the amplification process.

Statistical Analysis

All analyses were conducted using the SAS statistical software (SAS Institute, Cary, NC). The means and standard deviations of anthropometric and fitness data were stratified by case and control status, and two-tailed independent samples t-tests were performed to detect differences between groups. Mantel-Haenszel x2 test and Fisher’s exact test with 2 df were used to test differences in Gly482Ser genotype frequencies between groups. The association between genotype and V˙O2 max (for the athlete, fit, and unfit groups combined) was tested using linear regression models (PROC REG in SAS), where genotype was the predictor variable and V˙O2max was the continuous trait outcome.

RESULTS

Participant characteristics stratified by case and control status are presented in Table 1. Cases were young [mean age: 26.8 (3.8) yr], normal weight, Spanish male athletes (n = 104). Controls were unfit, middle-aged [mean age: 49.1 (8.3) yr] men selected from an existing population-based longitudinal study of UK Caucasians (n = 100) who were free of electrocardiographic abnormalities and other serious health ailments. As demonstrated by the characteristics in Table 1, controls were older, had higher body mass index [20.6 (1.5) vs. 27.6 kg/m2 (3.9); P < 0.0001] and were less fit than cases [73.4 (5.7) vs. 29.4 ml·kg−1·min−1 (3.8); P < 0.0001] (P < 0.0001). In cases and unfit controls, the Gly482Ser genotype met Hardy-Weinberg expectations (P > 0.05 in both groups tested separately).

Table 1. Participant characteristics stratified by case-control status

<table>
<thead>
<tr>
<th>Trait</th>
<th>Cases: Spanish (n = 104)</th>
<th>Fit Controls: UK (n = 100)</th>
<th>Unfit Controls: UK (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26.8 (3.8)</td>
<td>46.2 (8.2)</td>
<td>49.3 (8.1)*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176.5 (6.1)</td>
<td>176.2 (6.6)</td>
<td>175.3 (6.1)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>64.2 (6.7)</td>
<td>80.6 (12.1)</td>
<td>85.0 (13.2)*</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>20.6 (1.5)</td>
<td>25.9 (3.4)</td>
<td>27.6 (3.9)*</td>
</tr>
<tr>
<td>V˙O2max, ml·kg−1·min−1</td>
<td>73.4 (5.7)</td>
<td>41.2 (6.2)</td>
<td>29.4 (3.8)*</td>
</tr>
<tr>
<td>V˙O2max, l/min</td>
<td>4.70 (0.6)</td>
<td>3.32 (0.7)</td>
<td>2.49 (0.5)*</td>
</tr>
</tbody>
</table>

V˙O2max, maximal oxygen uptake; BMI, body mass index; UK, United Kingdom. Student’s t-test for difference between cases and unfit controls: *P < 0.0001.

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In Mantel-Haenszel $\chi^2$ analyses, the frequency of the minor Ser482 allele was significantly lower in cases than in unfit controls (29.1 vs. 40.0%; $P = 0.01$) (Fig. 1). Owing to the small sample size, we also undertook Fisher’s exact test. In this analysis, the results remained statistically significant ($P = 0.037$). These models also remained statistically significant after the inclusion of “fit” controls, whose Ser482 allele frequency was 33.8% ($P = 0.01$ with 3 df). In post hoc regression models, we combined VO$_{2\text{max}}$ data from the athlete, fit, and unfit groups and tested whether VO$_{2\text{max}}$ differed by genotype. In these models, mean (SD) level of VO$_{2\text{max}}$ was highest in the Gly482Gly genotype group [$n = 122$; 3.7 l/min (SD 1.1)], moderate in the Gly482Ser genotype group [$n = 154$; 3.4 l/min (SD 1.1)], and lowest in the Ser482Ser genotype group [$n = 27$; 1.7 l/min (SD 1.1)] ($P = 0.003$).

To assess the possibility of genetic stratification between Spanish and UK Caucasians, which could confound the association between genotype and endurance capacity, we also compared Gly482Ser genotype frequencies in two reference populations of Spanish ($n = 164$) and UK Caucasian men ($n = 381$) who were unselected for their level of fitness. Allele frequencies in both groups met Hardy-Weinberg expectations ($P > 0.05$ in both groups tested separately). In Mantel-Haenszel $\chi^2$ analyses, Ser482 allele frequencies did not differ significantly (36.9 vs. 37.5%, $P = 0.83$), suggesting that confounding by genetic stratification is unlikely to explain the association between Gly482Ser genotype and endurance capacity.

**DISCUSSION**

The present study compared the allele frequencies at the Gly482Ser locus of the PPARGC1A gene in elite-level endurance athletes and unfit controls. In athletes, the Gly482 allele was significantly more common than in unfit controls. Given that VO$_{2\text{max}}$ is a major determinant of endurance performance, and the unfit controls in the present study are defined by their low level of VO$_{2\text{max}}$, our data indicate that the presence of the Ser482 allele, in conjunction with other environmental and genetic factors, predicts aerobic capacity. This finding is interesting from the perspective of physical performance but may also have relevance in identifying individuals who, by virtue of their genetic predisposition to low cardiorespiratory fitness, may be at increased risk when sedentary of related disorders such as Type 2 diabetes. Thus, because aerobic capacity can be increased through exercise training, knowledge of the genotypes that predict those with low fitness presents an opportunity for targeted diseases prevention.

PPARGC1A mRNA is expressed predominantly in tissues with high metabolic activity, the majority of which are rich in mitochondria. These include heart, skeletal muscle (during exercise), brown fat, kidney, liver, and brain, and other tissues of low metabolic activity such as white adipose (29, 35). Through coactivation of the OXPHOS genes, PPARGC1A transiently controls glucose transportation through SLC2A4 regulation, and lipid and glucose oxidation through the regulation of the PPARs $\alpha$, $\delta$, and $\gamma$ (2). PPARGC1A also chronically modulates muscle oxidative capacity. This is achieved primarily via the coactivation of the nuclear respiratory transcription factors (NRF-1 and NRF-2), cytochrome-c oxidase 4 (COX4), and the mitochondrial transcription factors (mtTFA, mtTFB1, mtTFB2), which in a coordinated manner powerfully induce mitochondrial biogenesis (36). Inactivation of this signaling cascade is likely to result in attenuation of oxidative capacity and disordered metabolism that manifests as an insulin-resistance-like syndrome (8).

In human Type 2 diabetic skeletal muscle, OXPHOS genes are coordinately downregulated (23), and in morbid obesity PPARGC1A mRNA is threefold lower in subcutaneous and omental adipose tissue by comparison with tissue of lean controls (29). However, because data from these studies are cross sectional, one cannot determine whether downregulation of OXPHOS genes is a cause or consequence of diabetes and obesity.

In humans, a single 2-min bout of exhaustive exercise corresponds with increased PPARGC1A mRNA expression (7-10-fold), which peaks at around 2 h (26). Furthermore, by restricting blood flow to the exercising limb, PPARGC1A mRNA overexpression is prolonged (to ~6 h) (25), suggesting that metabolic perturbation influences PPARGC1A mRNA expression. Short et al. (30) recently reported 1.5-fold increases in PPARGC1A mRNA levels in men and women after a 16-wk exercise training program consisting of stationary cycling (3–4 sessions/wk at 70–80% maximum heart rate), and Russell et al. (28) reported chronic increases in PPARGC1A mRNA expression (2.7-fold) after 6 wk of running training. Moreover, these changes in expression are independent of increased type 1 fiber quantity or preferential fiber-type switching. However, others have been unable to detect changes in PPARGC1A expression after 9 days of cycle ergometry (60 min/day) (34) or after a single bout of one-legged knee extension exercise (25). The fact that PPARGC1A expression levels increase after exercise is important, because the presence of functional genetic variation at this locus could, therefore, result in differential levels of expression after exercise, which would depend on genotype.

Several studies have reported an increased frequency of the Ser482 allele in Type 2 diabetic patients (9, 15, 24). Given the metabolic characteristics of PPARGC1A and the manner in
which exercise training modulates its expression, it is plausible that variation at the Gly482Ser locus modifies the protective effect of physical activity on risk of diabetes-related traits. For example, in a recent study of Danish adults (20), the Ser482 allele was inversely associated with PPARGC1A mRNA level and VO2 max. Furthermore, we have previously described the interaction between physical activity and the Gly482Ser variant on VO2 max (10).

A limitation of case-control and cross-sectional studies, such as the present study and those reported elsewhere, is that they do not provide evidence that, with training, adaptation in VO2 max differs by genotype. For this purpose, randomized clinical trials are necessary. An additional potential limitation of this study is that cases and unfit controls differ by ethnicity, which may correspond with population-specific genotype frequencies. If this is the case, then the associations that we report could represent the relationship between genotype and ethnicity, and not an association between Gly482Ser genotype and endurance capacity, as hypothesized. However, we tested the potential for this form of confounding in two reference samples of Spanish and UK Caucasians that were not defined by level of fitness. In this analysis, genotype frequencies were virtually identical in Spanish and UK Caucasians, suggesting that confounding by genetic stratification is unlikely to explain the association we report between genotype and endurance capacity. A further potential limitation could involve confounding due to a survivor effect. This would occur if the Gly482 allele was associated with mortality at a young age, because the younger group in the present study (i.e., the athletes) would be characterized by a higher frequency of the Gly482 allele by comparison with the older group (i.e., the unfit controls). However, we tested the association between the Gly482Ser genotype and age in the MRC Ely cohort, which includes individuals of a similar age as the Spanish athletes, and found no evidence of association (data not shown), suggesting that a survivor effect is unlikely to explain our observations.

In summary, our data indicate that a lower frequency of the Ser482 allele is associated with a higher aerobic capacity. The frequency of this allele in unfit population controls (40%) is similar to that reported previously in Danish diabetic patients (37%) (9). By contrast, fit controls in our study have a lower Ser482 allele frequency (33%), and elite-level athletes have the lowest allele frequency (29%) of all groups. Thus, given that a low level of cardiorespiratory fitness is a strong risk factor for diabetes, it is possible that fitness mediates the previously reported relationship between Gly482Ser genotype and diabetes.

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GRANTS

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