PGC-1α Genotype Modifies the Association of Volitional Energy Expenditure with VO₂max

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ABSTRACT

FRANKS, P. W., I. BARROSO, J. LUAN, U. EKELUND, V. E. F. CROWLEY, S. BRAGE, M. S. SANDHU, R. W. JAKES, R. P. S. MIDDELBERG, A.-H. HARDING, A. J. SCHAFFER, S. O’RAHILLY, and N. J. WAREHAM. PGC-1α Genotype Modifies the Association of Volitional Energy Expenditure with VO₂max. Med. Sci. Sports Exerc., Vol. 35, No. 12, pp. 1998–2004, 2003. Sedentary lifestyles are increasingly common and result in low cardiorespiratory fitness (VO₂max), a well-established predictor of early mortality and coronary heart disease (CHD). Adaptation in VO₂max after exercise training varies considerably between people. Because there are hereditary components to fitness, it is likely that genetic factors explain some of this variability. PPARG1 (PGC-1α) coactivates genes involved in energy transduction and mitochondrial biogenesis. Transgenic mouse data demonstrate that overexpression of PGC-1α mRNA increases endurance capacity by transformation of nonoxidative to oxidative skeletal muscle tissue. Other murine studies demonstrate that exercise increases PGC-1α mRNA expression. Purpose: To explore whether a candidate polymorphism in the PGC-1α gene modifies the association between physical activity energy expenditure (PAEE) and predicted VO₂max (VO₂max,pred).

Method: We examined whether the Gly482Ser polymorphism of PGC-1α modified the relationship between objectively measured PAEE and VO₂max,pred in a population-based sample of 599 healthy middle-aged people. PAEE was assessed using individual calibration with 4 days of heart rate monitoring. VO₂max,pred was measured during a submaximal exercise stress test on a bicycle ergometer.

Results: Homozygosity at the Ser482 allele was found in 12.7% of the cohort, whereas 38.9% and 48.4% carried the Gly482Gly and homozygous for the Ser482 allele (β = 12.03; P < 0.00001) compared with carriers of the Gly allele (β = 5.61; P < 0.00001). In a recessive model for the Ser482 allele, the interaction between PAEE and genotype on VO₂max,pred (L·min⁻¹) was highly significant (P = 0.009). Conclusion: Our results indicate that Ser482 homozygotes may be the most capable of improving cardiorespiratory fitness when physically active, and that Gly482Ser may explain some of the between-person variance previously reported in cardiorespiratory adaptation after exercise training. Key Words: PHYSICAL ACTIVITY, FITNESS, GENE-ENVIRONMENT INTERACTION, POLYMORPHISM

Western populations are becoming increasingly sedentary. Low cardiorespiratory fitness (VO₂max) is associated with physical inactivity and is an established predictor of all-cause mortality and metabolic disease (30,32,33). Although regular exercise improves VO₂max, the degree of improvement varies considerably between individuals (5). Cardiorespiratory fitness segregates within families (4), suggesting that hereditary factors influence cardiorespiratory trainability. Thus, individuals with a genetic predisposition to improved cardiorespiratory fitness after exercise may be those who benefit most from preventative strategies designed to increase volitional energy expenditure.

Peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1α) is a strong candidate gene for cardiorespiratory fitness. PGC-1α mRNA is expressed predominantly in hyper-energetic tissues, the majority of which are rich in mitochondria. These include heart, exercising skeletal muscle, brown fat, kidney, liver, and brain (25). One of the roles of PGC-1α is to stimulate mitochondrial biogenesis. This is achieved by coactivating the nuclear respiratory transcription factors NRF-1 and NRF-2, which modify the expression of many mitochondrial proteins (34).

Functional data indicate that ectopic expression of PGC-1α in muscle cell lines results in a powerful induction of mitochondrial biogenesis and of genes involved in both nuclear and mitochondrial energy transduction pathways (34). Several murine studies have shown that overexpression of PGC-1α is associated with an enhanced oxidative capacity of skeletal muscles and prolonged time to fatigue during exercise (2,15,17,28). In a recent study by Lin et al.
(20), muscle-specific transgenic overexpression of PGC-1α in mice was associated with an increased oxidative capacity of skeletal muscle tissue compared with the tissue of non-transgenic litter mates. A subsequent study by Baar et al. (2) demonstrated that in nontransgenic Wistar rats PGC-1α mRNA expression increased significantly after low-intensity bouts of swimming lasting around 3 h.

Given the known biology of the PGC-1α gene and the product it encodes, it is plausible that variants in this gene may modulate the manner in which human skeletal muscle responds to aerobic stress. The PGC-1α gene was selected based on previously reported biological function (2,15,17,28), and the Gly482Ser polymorphism was chosen because of its reported sequence variation in normal, diabetic, and obese cohorts (10,13,17,23). The previously described functional role of PGC-1α allowed us to target our examination of interaction on the link between this gene and physical activity, thus reducing the number of statistical tests undertaken and preserving statistical power. Therefore, the aim of this study was to examine whether a common polymorphism (Gly482Ser) within the PGC-1α gene modified the association between physical activity energy expenditure (PAEE) and VO₂max_pred in a large population-based cohort study where objective assessments of PAEE and VO₂max_pred are available. In this study, we made assessments of PAEE by undertaking individual calibration of heart rate against energy expenditure at rest and during exercise, combining this with continuous heart rate monitoring during 4 d of free-living (HR flex). This method has previously been validated against whole-body calorimetry (6,21), and has proven to be highly reliable (31). The availability of objectively assessed PAEE in a large population-based sample provides an ideal opportunity to search for gene-physical activity interactions.

METHODS

Selection of the participants and metabolic tests. The volunteers were all participants in the Medical Research Council (MRC) Ely Study, a continuing population-based cohort study in Ely, Cambridgeshire, the design of which has been described previously (31). The original sample of 1122 individuals without known diabetes were recruited between 1990 and 1992 at random from a population-based sampling frame consisting of all people in Ely, Cambridgeshire, aged 40–65 yr in 1990. From 1994 to 1997, a second examination was performed at a 4.5-yr interval in all individuals who did not have diabetes by WHO criteria at baseline (N = 1071). Twenty participants had died in the interim, and 937 of the remaining volunteers attended for the second examination. Of these, complete anthropometric, genetic, and energy expenditure data were available for a total of 599 people. These individuals constituted the sample for this study.

Volunteers attended the clinic at 8:30 a.m. having fasted since 10 p.m. the previous evening. After an explanation of laboratory procedure, all participants provided written informed consent. Smoking status was assessed via questionnaire and coded as “never-smoker,” “ex-smoker,” or “current-smoker.” Body fat percentage was calculated using a standard impedance technique (Bodystat, Isle of Man). Height and weight were measured in light clothing. Body circumferences were measured in duplicate using a metal tape. Glucose, insulin, and nonesterified fatty acid (NEFA) levels were assessed using standard techniques before and during a 75-g oral glucose tolerance test (22,30,31). Ethical permission for the study was granted by the Cambridge Local Research Ethics Committee.

Assessment of resting and exercise oxygen consumption-heart rate relationship. The protocol for undertaking the individual calibration between heart rate and energy expenditure has been reported previously (30,31). This method correlates to a high degree with energy expenditure assessed through the gold standard techniques of doubly labeled water during field trials (21) and whole-body calorimetry (6). The oxygen consumption-heart rate relationship was assessed at rest with the participant lying and then seated, using an oxygen analyser calibrated daily with 100% nitrogen and fresh air as standard gases (PK Morgan Ltd, Chatham, Kent, UK). Participants bicycled on a cycle ergometer at several different workloads to provide the slope and the intercept of the line relating energy expenditure to heart rate. Each participant cycled at 50 rpm, and the workload was progressively increased from 0 W, through 37.5 W, 75 W, and 125 W in stages each lasting 5 min. At each workload, three separate readings were made of heart rate, minute volume, and expired air oxygen concentration. The 125-W level was only undertaken if the heart rate had not reached 120 bpm by the end of the 5 min at 75 W. The oxygen concentration in the expired air and minute volume data were used to calculate oxygen consumption after correction for standard temperature and pressure. Energy expenditure (kJ·min⁻¹) was calculated at each time point as oxygen consumption (mL·min⁻¹) × 20.35 (11). Mean resting energy expenditure was taken as the average of the lying and sitting values. The slope and intercept of the least squares regression line of the exercise points was calculated. Flex heart rate was calculated as the mean of the highest resting pulse rate and the lowest on exercise. This point was used in the analysis of heart rate data to discriminate between rest and exercise. Below this point, energy expenditure was assumed to be equivalent to rest. Energy expenditure above this level was predicted from the slope and intercept of the regression line calculated during the exercise test. VO₂max_pred was measured from the linear regression as predicted oxygen consumption at maximum heart rate (220 — age) and is expressed in the results as volume per kilogram (body weight) per minute. The volunteers wore the heart rate monitor (Polar Electro Ltd., Kempele, Finland) continuously during the waking hours over the following 4 d. Heart rate readings were directly downloaded into a computer via a serial interface, and the individual calibration data were used to predict min energy expenditure for each person. Sleeping energy expenditure was calculated as 95% of basal metabolic rate (BMR), where this was derived from published prediction equations (14,26). Phys-
rical activity energy expenditure (PAEE), which is the ratio of total energy expenditure to BMR, was computed for each day and averaged over the 4-d period.

**Genetic analyses.** Previous studies in humans that have examined the relationship between the Gly482Ser genotype and diabetes, and its intermediate traits, have suggested that carriers of the Ser482 allele may be predisposed to an increased risk of metabolic morbidity (10,13,17,23). In a transgenic mouse study reported by Lin et al. (20), the authors suggest that in humans this predisposition may be related to lipid and glucose oxidation in those carrying certain functional mutations within the PGC-1α gene (20). Given these observations, and that the major allele of a gene is likely to be the wild-type allele, we hypothesized that the homozygous Ser482Ser genotype should be compared with the individuals carrying at least one Gly482 allele.

DNA was extracted from blood samples using standard protocols (16). PGC-1α sequence NM_01326 was obtained from locuslink (http://www.ncbi.nlm.nih.gov/LocusLink). In summary, genomic DNA from participants was randomly preamplified in a 50 μL PEP (primer extension preamplification) reaction using methods previously described (36). The PEP amplified DNA samples were PCR amplified in 8-μL reactions with primers flanking the Gly482Ser site (10). Unincorporated dNTP 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 (37). At the end of the reaction, the samples were held at 4°C. Single-base primer extension reactions were performed as previously described by Chen et al. (7), and allele detection was performed by measuring fluorescence polarization on an LJM Analyst fluoroscent reader (Molecular Devices). Genotyping was performed using an adaptation of the fluorescence polarization template directed incorporation (FP-TDI) method (7).

**Statistics.** The means and standard deviations of anthropometric, fitness, and energy expenditure data were stratified by genotype of the Gly482Ser polymorphism. Two-tailed independent samples t-tests were performed to detect differences between Gly482Ser genotypes for each variable. Multiple linear regression analyses were performed using SPSS (release 10.0.5) to assess the interaction between Gly482Ser genotypes and PAEE on VO\textsubscript{max, pred}. PAEE was entered into the model as a continuous variable. The main interaction analyses were undertaken in a recessive model for the Ser482 allele and were adjusted for age, sex, body mass (kg), smoking, fasting insulin, 2-h glucose, and NEFA AUC.

**RESULTS**

All analyses were initially performed stratified by sex. However, because the directions of effect for Gly482Ser genotypes were the same for men and women, data were combined. Genotype frequencies were 38.9% (Gly482Gly), 48.4% (Gly482Ser), and 12.7% (Ser482Ser). The Gly482Ser genotype did not deviate significantly from Hardy-Weinberg equilibrium ($P > 0.05$), indicating that the population under investigation is not biased in the distribution of allele frequencies for the Gly482Ser polymorphism and that nonrandom mating, first generation mutations, and nonrandom migration are unlikely to be prevalent. Interaction analyses were initially undertaken in a codominant model, where the Ser482Ser genotype was treated as the baseline category ($P$ for interaction 0.018). However, because the regression coefficients for the Gly482Gly and Gly482Ser groups did not differ, these genotypes were combined and compared with the Ser482Ser genotype to improve the model’s statistical power. Table 1 shows participant characteristics stratified by genotype in a recessive model for the Ser482 allele. In sex-combined analyses that were both unadjusted and adjusted for age, sex, body fat %, and smoking, people who carried the common Gly482 allele did not differ significantly from those homozygous for the Ser482 allele for resting energy expenditure, PAEE, VO\textsubscript{2max, pred}, 2-h glucose, fasting insulin, or any anthropometric parameters examined. However, NEFA AUC was higher in Ser482 homozygotes as compared with Gly482 allele carriers ($P = 0.009$). Table 2 shows Pearson correlation coefficients stratified by genotype. BMI, BF%, REE, PAEE, and fasting insulin were all significantly correlated with VO\textsubscript{2max, pred} (L·min\textsuperscript{-1}) in Gly482 allele carriers. BF%, REE, PAEE, and NEFA AUC were correlated with VO\textsubscript{2max, pred} in Ser482 allele homozygotes.

The relationship between PAEE and VO\textsubscript{2max, pred} (mL·kg\textsuperscript{-1}·min\textsuperscript{-1}) was highly significant in this study ($\beta =$

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<th>2-h glucose (mmol·L\textsuperscript{-1}) 0.23</th>
<th>Smoking (never/ex/current)† 0.23</th>
<th>REE (kJ·kg\textsuperscript{-1}·min\textsuperscript{-1}) 0.23</th>
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<td>Fasting insulin (pmol·L\textsuperscript{-1})‡ 0.23</td>
<td>2 hr glucose (mmol·L\textsuperscript{-1})‡ 0.23</td>
<td>Smoking (never/ex/current)‡ 0.23</td>
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6.60, $P < 0.0001$), as elsewhere (9). However, in stratified analyses the association was stronger in Ser482 allele homozygotes ($\beta = 12.03$, $P < 0.00001$) compared with Gly482 allele carriers ($\beta = 5.61$, $P < 0.00001$). In multiple-regression analysis (Table 3), the adjusted interaction of PGC-1$\alpha$ and PAEE on VO$\textsubscript{2max, pred}$ (L·min$^{-1}$) was statistically significant ($P = 0.009$). Figure 1 shows the interaction between PAEE and Gly482Ser genotype on VO$\textsubscript{2max, pred}$ in continuous form and assuming a recessive effect of the Ser allele. From Figure 1, it is evident that the relationship between PAEE and VO$\textsubscript{2max, pred}$ is significantly stronger in Ser482 allele homozygotes compared with Gly482 allele carriers. Furthermore, despite similar levels of maximal oxygen uptake between genotypes at low activity levels, due to a significantly stronger association between PAEE and VO$\textsubscript{2max, pred}$ in Ser/Ser homozygotes compared with Gly allele carriers, at moderate to high activity levels oxygen uptake is considerably greater in Ser homozygotes.

Recent findings from Muller and coworkers (23) indicate that the association between Gly482Ser and Type 2 diabetes reported in previous studies (10,13,17) may be due to altered $\beta$-cell function and capacity for glucose and lipid oxidation. To assess whether these factors could confound the interaction between PAEE and Gly482Ser on VO$\textsubscript{2max, pred}$ we conducted an additional analysis where 2-h glucose, fasting insulin and NEFA area under the 2-h curve (AUC) were adjusted for (Table 3 and Fig. 1). Although in univariate analysis, Ser482 homozygotes had significantly higher NEFA AUC as compared with Gly482 allele carriers ($\beta = 0.06$; $P = 0.009$), adjusting for insulin, glucose, and NEFA made no difference to the magnitude of the interaction or its level of significance ($\beta = -0.42$, $P = 0.007$ vs $\beta = -0.42$, $P = 0.009$, basic and complex models, respectively).

**DISCUSSION**

This study examined the interaction between a common polymorphism (Gly482Ser) in the peroxisome proliferator-activated receptor $\gamma$ coactivator 1 gene (PGC-1$\alpha$) and physical activity energy expenditure (PAEE) on predicted maximum oxygen uptake (VO$\textsubscript{2max, pred}$) in a large free-living UK population. PAEE was strongly positively associated with VO$\textsubscript{2max, pred}$ in all genotypes. However, this relationship was significantly stronger in Ser482 allele homozygotes compared with Gly482 allele carriers (Gly482Gly + Gly482Ser). Ser482Gly homozygotes may have the greatest potential to increase VO$\textsubscript{2max}$ most when engaging in physically active lifestyles and may consequently derive superior health benefits compared with Gly482 carriers.

It is unclear from existing evidence whether there is a causal relationship between polymorphisms in the PGC-1$\alpha$ gene and metabolic disease. The first study published on PGC-1$\alpha$ in humans reported an odds ratio of 1.34 ($P = 0.0007$) for risk of diabetes in Danish carriers of the Ser482 allele (10). Esterbauer and colleagues (13) found no association in Austrian participants between PGC-1$\alpha$ haplotypes, estimated from Gly482Ser and $+$2962A/G genotypes, and diabetes or intermediate traits when analyses were conducted using sex-combined data. When stratified by sex, however, associations were observed between PGC-1$\alpha$ haplotype and BMI ($P = 0.006$), waist circumference ($P = 0.012$), body fat mass ($P = 0.007$), and HDL-C ($P = 0.028$) in women only. In a study of older Japanese men and women, Hara and colleagues (17) observed a difference in the frequencies of PGC-1$\alpha$ haplotypes, constructed from the Thr394Thr and Gly482Ser genotypes, between diabetics and nondiabetics ($P = 0.00003$). Hara et al. also reported associations between the Ser482 allele and fasting insulin level ($P = 0.018$), and insulin resistance assessed through the homeostasis model ($P = 0.027$) in nondiabetic controls. In a study of French diabetics and nondiabetic controls, Lacquemant et al. (19) found no association between the Gly482Ser polymorphism and diabetic status. In the only study thus far to assess the relation-
ship between PGC-1α variants and hypertension, Obkerkofler and collaborators (24) found that in Austrian men Ser482 homozygosity was associated with hypertension when compared with Gly482 homozygotes (OR = 2.38; \( P = 0.004 \)) but found no association in women. In the most recent study of PGC-1α and diabetes, Muller and colleagues (23) found no association between the Gly482Ser genotype and diabetic status or with BMI in Pima Indians. However, young adult nondiabetic carriers of the Ser482 allele had higher 30-min plasma insulin (\( P = 0.04 \)), a higher level of lipid oxidation (\( P = 0.020 \)), higher acute insulin response (\( P = 0.0001 \)), and lower plasma NEFA levels (\( P = 0.02 \)). Ser482 allele carriers also had smaller adipocytes compared with Gly482 homozygotes. Assuming that the PGC-1α gene interacts with environmental factors, it is possible that the heterogeneity evident in the associations reported so far between variants in the PGC-1α gene and metabolic dysfunction may be due to varying environmental exposures between cohorts. Given the functional evidence relating PGC-1α with aspects of energy transduction and substrate oxidation (2,15,28), it is possible that physical activity is an important environmental exposure that modifies the effects of PGC-1α on metabolism.

PGC-1α plays an important role in energy homeostasis (34,35) and modifies oxidative capacity in rodents (2,15,17,28). Given also the genotypic association with Type 2 diabetes, in humans, (10,13,17,23). PGC-1α is a viable candidate gene for modified metabolic adaptation through a physically active lifestyle. The data presented in this paper describe for the first time in humans the modification of the relationship between physical activity and cardiorespiratory fitness by a common PGC-1α variant (Gly482Ser). The oxidative capacity of muscle is a key determinant of maximum oxygen uptake. Therefore, \( \dot{V}O_2 \) per unit body mass is likely to be higher in people with a greater ratio of slow- to fast-twitch muscle fibers. PGC-1α is a transcriptional coactivator of nuclear receptors and other transcription factors that regulate aspects of metabolism and energy expenditure (30,31). One of the nuclear transcription factors that PGC-1α coactivates (PPARα) has recently been shown to modify the effect of exercise training on left-ventricular hypertrophy (18), which may be one mechanism through which PGC-1α modifies the capacity for oxygen uptake. However, the strongest evidence published to date that illustrates the role PGC-1α may play in modifying oxidative capacity comes from murine models. A recent study of transgenic mice demonstrated that PGC-1α is highly expressed in oxidative skeletal muscle tissue, that overexpression of PGC-1α increases the conversion rate of fast- to slow-twitch muscle fiber after exercise and that overexpression of PGC-1α mRNA increases time to fatigue of the hind limb during electrical stimulation (17). Other rodent data show that long-duration low-intensity aerobic exercise increases the expression of PGC-1α mRNA (2,15,28). Because sustained aerobic exercise training also causes the conversion of nonoxidative to oxidative muscle tissue in humans (3), it is possible that variants in the PGC-1α gene modify the degree to which the oxidative capacity of skeletal muscle changes after repetitive sustained physical activity. This effect may be indicated by the PAEE-adjusted variation across PGC-1α genotypes in maximal oxygen uptake measured during an exercise stress test. However, because muscle fiber conversion occurs mainly after intense aerobic exercise training (3), which is not characteristic of the level of activity seen in the MRC Ely cohort, it is unlikely that this would be the main explanation for our findings.

In the recent study in Pima Indians (23), Muller and colleagues speculate that the differential levels of lipid and glucose oxidation they observed between Gly/Gly homozygotes and Ser allele carriers may be mediated by coactivation of the PPARs α and γ. Considering the known biology of PGC-1α, this explanation is highly plausible (27,35). However, given that adjusting our interaction models for glucose, insulin, and NEFA made no difference to either the magnitude of the interaction coefficient or its level of significance, it is unlikely that alterations to β-cell function or glucose or lipid oxidation explain the interaction between Gly482Ser and PAEE that we observed. In contrast to Muller et al.’s findings, we observed higher NEFA AUC in Ser482 homozygotes, whereas Muller et al. observed higher NEFA levels in Gly482 homozygotes. This disparity may be explainable by genetic heterogeneity due to ethnicity, between-cohort age differences, or by the possibility that either our and/or Muller et al.’s findings are false positives. Alternatively, given the gene-environment interaction we observed in the present study, it is also plausible that these differences are attributable to the lower levels of physical activity energy expenditure, which is characteristic of Pimas (12) as compared with participants in our study.

The power to detect gene-environment interactions in analyses of quantitative traits is a product of sample size, the frequency of the minor allele, the precision with which the environmental exposure and outcomes are measured, and the between-genotype difference in the size of effect. The sample size in this study is large compared with most other studies where an interaction between genetic factors and physical activity has been considered, and the frequency of the Ser482Ser genotype was approximately 13%. Most studies of this nature have used questionnaires to assess physical activity level. To our knowledge, only one other interaction study has been published to date where objective assessment of physical activity was made (22). The association between physical activity and maximum oxygen uptake was positive in all Gly482Ser genotypes. However, the slope of the relationship in Ser482 allele homozygotes was significantly stronger than in Gly482 allele carriers. In combination, these factors ensure that the power to detect interaction in this study is likely to be greater than most other studies where gene-environment interactions were examined. The likelihood of reporting false-positive results in this study was reduced by the use of a priori biologically plausible hypotheses (1).

The cross-sectional nature of this study limits inference about causality. In this study, we assumed that physical
activity precedes cardiorespiratory fitness on the causal pathway. However, it is possible that low fitness predisposes some individuals to be sedentary. Therefore, the relationship between PAEE and VO2max may not always be unidirectional. However, the main aim of this study was to examine the potential for effect modification by the Gly482Ser polymorphism. Given the evidence from functional data regarding the role of PGC-1α in energy transduction, it seems improbable that this gene modifies the effect of fitness on activity level. Therefore, even if in some cases VO2max precedes PAEE on the causal pathway, this would simply weaken the interaction between PAEE and genotype, and would not bias or confound our results.

Volunteers for this study were randomly selected from a large population-based sampling frame. The anthropometric and fitness characteristics of this population are comparable to other national representative samples where these factors have been assessed (8, 29). The allocation of days on which volunteers attended our laboratory and on which energy expenditure was assessed was random and involved week and weekend days. We have previously demonstrated that there is no overall difference in energy expended on week and weekend days (31). In view of these points, it is unlikely that selection bias could explain our results.

This is to our knowledge the only study in humans that has examined the role of a polymorphism in the PGC-1α gene in modifying the relationship between physical activity energy expenditure and maximum oxygen uptake. Although our data indicate that individuals homozygous for the Ser482 allele may have the greatest capacity to improve fitness when physically active, this hypothesis will require testing in an etiological trial in order to fully discern causality. Furthermore, even if this is true, it does not necessarily follow that the higher VO2max after training in Ser482 homozygotes corresponds with a decreased susceptible to metabolic disease compared with Gly allele carriers. This is because VO2max reflects capacity for substrate oxidation and can be both an independent exposure and an outcome of other exposures. Higher VO2max at a given physical activity level could reflect a training response, and this is often how it is perceived. However, depending on the region of inhibition, it could also reflect oxidative inefficiency within muscle, which could be characterized by a higher VO2max at a given workload. Thus, because VO2max is a multidimensional phenotype, it may be necessary to comprehend how these dimensions vary across genotypes, and how such variation relates with diabetes and its intermediate traits. Understanding of this area is likely to be advanced through the examination of extreme phenotype cohorts, such as diabetics, obese, and elite endurance athletes. These studies may also involve the precise characterization of muscle morphology and measurements of substrate oxidation. Greater understanding of the biological mechanisms that mediate the effect of the PGC-1α gene on oxygen uptake kinetics and modify the conditioning effects of physical activity in humans may help improve the effectiveness of physical activity as a preventive strategy for metabolic disease.

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REFERENCES


