Impact of the peroxisome proliferator activated receptor- γ coactivator-1 β (*PGC-1 β*) Ala203Pro polymorphism on in vivo metabolism, *PGC-1 β* expression and fibre type composition in human skeletal muscle

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Abstract

Aims/hypothesis Peroxisome proliferator activated receptor- γ coactivator-1 β (*PGC-1 β* , also known as *PPARGC1B*) expression is reduced in skeletal muscle from patients with type 2 diabetes mellitus and in elderly subjects. Ala203Pro, a common variant in the *PGC-1 β* gene is associated with obesity. The aim of this study was to investigate whether the *PGC-1 β* Ala203Pro polymorphism influences the age-related decline in skeletal muscle *PGC-1 β* expression, in vivo metabolism and markers for muscle fibre type composition. **Materials and methods** The *PGC-1 β* Ala203Pro polymorphism was genotyped in 110 young (age 28.0 ± 1.9 years) and 86 elderly (age 62.4 ± 2.0 years) twins and related to muscle *PGC-1 β* expression, in vivo metabolism and markers for fibre type composition. **Results** Insulin-stimulated non-oxidative glucose metabolism (NOGM; $p=0.025$) and glycolytic flux rate (GF; $p=0.026$) were reduced in young Ala/Ala carriers compared

with carriers of a 203Pro allele. In addition, a regression analysis, correcting for covariates, showed that the *PGC-1 β* 203Pro allele was positively related to insulin-stimulated NOGM and GF in the young twins. While muscle expression of *PGC-1 β* was reduced in elderly compared with young carriers of the Ala/Ala genotype ($p \leq 0.001$), there was no significant age-related decline in *PGC-1 β* expression in carriers of the 203Pro allele ($p \geq 0.4$). However, a regression analysis, correcting for covariates, showed that only age was significantly related to muscle *PGC-1 β* expression. Finally, *PGC-1 β* expression correlated positively with markers for oxidative fibres in human muscle.

Conclusions/interpretation This study suggests that young carriers of a *PGC-1 β* 203Pro allele have enhanced insulin-stimulated glucose metabolism and may be protected against an age-related decline in *PGC-1 β* expression in muscle.

Keywords Fibre type · Gene expression · In vivo metabolism · *PGC-1 β* · Polymorphism · *PPARGC1B* · Skeletal muscle

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Abbreviations

DZ	dizygotic
ERR	oestrogen receptor-related receptor
GEE	generalised estimating equations
GF	glycolytic flux
LBM	lean body mass
MZ	monozygotic
NOGM	non-oxidative glucose metabolism
PGC-1 β	peroxisome proliferator activated receptor- γ coactivator-1 β
PPAR	peroxisome proliferator activated receptor

Introduction

Peroxisome proliferator activated receptor (PPAR)- γ coactivator-1 β (*PGC-1 β*) is part of a small family of transcriptional co-activators that also includes PPAR- γ coactivator-1 α (*PGC-1 α*) and PGC-1 related coactivator (PRC). These co-activators influence metabolic pathways in muscle, liver, adipose tissue, pancreas and heart by co-activating a number of transcription factors including PPAR γ , PPAR α , hepatocyte nuclear factor 4 alpha (HNF4 α), nuclear respiratory factor 1 (NRF-1), myocyte enhancer factor 2 (MEF2), oestrogen receptor-related receptor (ERR) α and forkhead box O1 (FOXO1) [1].

Compared with healthy control subjects, patients with type 2 diabetes mellitus have reduced expression of *PGC-1 α* (also known as *PPARGC1A*) and *PGC-1 β* (also known as *PPARGC1B*), as well as of a set of genes involved in oxidative phosphorylation in skeletal muscle [2, 3]. We originally showed that a common variant in the *PGC-1 α* gene, Gly482Ser, is associated with increased risk of type 2 diabetes mellitus as well as with altered lipid and glucose metabolism [4]. Furthermore, we demonstrated that combinations of genetic and environmental factors influence the *PGC-1 α* and *PGC-1 β* expression in human muscle [5]. Interestingly, the *PGC-1 α* Gly482Ser polymorphism was found to accelerate an age-related decline in the expression of both *PGC-1 α* and *PGC-1 β* in muscle [5]. In a recent study, we identified a polymorphism in the *PGC-1 β* gene, Ala203Pro, where the common allele Ala203 conferred an increased risk of obesity [6]. In addition, recent studies in rodents suggest that *PGC-1 β* influences the composition of fibre types in skeletal muscle [7–9].

The present study aimed to investigate whether this common polymorphism in *PGC-1 β* , Ala203Pro, influences in vivo glucose and fat metabolism as well as *PGC-1 β* expression in muscle of young ($n=110$, age 28.0 ± 1.9 years) and elderly ($n=86$, age 62.4 ± 2.0 years) twins without known diabetes. We also investigated whether *PGC-1 β* relates to markers for fibre type composition in skeletal muscle of twins.

Materials and methods

Participants Participants were identified through the Danish Twin Register. Ninety-eight twin pairs (33 younger monozygotic [MZ]; 22 younger dizygotic [DZ]; 21 elderly MZ; 22 elderly DZ) without known diabetes were enrolled in the clinical examination. The clinical examination and characteristics of these twins have been described previously [5]. Informed consent was obtained from all subjects. The present study was approved by the regional ethical committees and conducted according to the Helsinki Declaration.

Analysis of PGC-1 β , myosin heavy chain (MHC)7, MHCIIa and MHCIIx/d mRNA levels in muscle Gene expression was analysed in 156 muscle biopsies of the vastus lateralis muscle taken in both the fasting state and after a 2 h hyperinsulinaemic–euglycaemic clamp ($40\text{ mU m}^{-2}\text{ min}^{-1}$) from 78 twin pairs (26 younger MZ; 18 younger DZ; 15 elderly MZ; 19 elderly DZ) using TaqMan Real-Time PCR with an ABI 7900 system (Applied Biosystems, Foster City, CA, USA) and assays-on-demand (all Applied Biosystems) for *PGC-1 β* (Hs00370186_m1), myosin heavy chain (*MHC*, also known as *MYH7*) (Hs00165276_m1), *MHCIIa* (Hs00430042_m1) and *MHCIIx/d* (Hs00428600_m1). The transcript quantity was normalised to that of cyclophilin A mRNA (4326316E; Applied Biosystems).

Genotyping The *PGC-1 β* Ala203Pro variant was genotyped using PCR restriction fragment length polymorphism analysis with the primers 5'-GTG GGG CTT TGT CAG TGA AT-3' and 5'-GGA CTC CTG GAG GCA TGG TG-3' and digestion with NlaIV. The restriction enzyme digests were separated on 4% agarose gels and visualised by ethidium bromide staining.

Statistical methods Because of the strong intrapair correlation of twin data, conventional tests of differences between variable (y) means are not valid. To correct for this dependence we used generalised estimating equations (GEE) methodology ($y=\alpha+\beta x$) to provide valid standard errors for the β coefficients [10, 11]. The β coefficient was estimated from all observations, whereas for calculation of the variance of β , each family was considered as one cluster.

To identify factors which were independently associated with the response variable, we used backwards regression analysis with a $p>0.05$ as the defining criteria for exclusion. In this analysis, too, we used generalised estimation equations (GEE) methodology to obtain valid tests.

Results

Clinical characteristics As presented previously, elderly twins were more obese and had reduced glucose disposal rate, storage and oxidation than younger twins [5].

Influence of the PGC-1 β Ala203Pro polymorphism on the age-related decline in muscle gene expression We have previously demonstrated that elderly twins have significantly lower mRNA levels of *PGC-1 β* in muscle than younger twins [5]. We demonstrate here that this age-related decline in muscle *PGC-1 β* expression might be influenced by the *PGC-1 β* Ala203Pro polymorphism (Fig. 1a,b). Both in the basal state (young 1.37 ± 0.09 vs

Fig. 1 The effect of the *PGC-1β* Ala203Pro polymorphism on the age-related decline in skeletal muscle gene expression in twins. The mRNA level of *PGC-1β* was measured in skeletal muscle of young (open bars) and elderly (closed bars) twins **a** before and **b** after a hyperinsulinaemic clamp and related to the *PGC-1β* Ala203Pro polymorphism. Results are expressed as the mean±SEM for Ala/Ala carriers ($n=80$ and $n=50$ for young and elderly, respectively) and carriers of a 203Pro allele, Ala/Pro + Pro/Pro ($n=8$ and $n=18$ for young and elderly, respectively). To correct for the lack of independence of twin data, all comparisons of mean differences between groups were performed using generalised estimation equations (GEE) methodology. **, $p<0.001$; #, $p=0.9$; †, $p<0.0001$; ‡, $p=0.4$

elderly 0.93 ± 0.07 ; $p=0.0007$) and in the insulin-stimulated state (young 2.51 ± 0.17 vs elderly 1.58 ± 0.12 ; $p=0.00004$), the elderly twins carrying the *PGC-1β* Ala203Ala genotype had lower *PGC-1β* gene expression than young Ala203Ala carriers. There was, however, no significant influence of age on muscle *PGC-1β* expression in carriers of a *PGC-1β* 203Pro allele, either in the basal (young 1.24 ± 0.16 vs elderly 1.21 ± 0.21 ; $p=0.9$) or in the insulin-stimulated state (young 2.41 ± 0.43 vs elderly 1.92 ± 0.24 ; $p=0.4$; Fig. 1a,b). We performed a regression analysis to test whether any of

the following factors influence skeletal muscle *PGC-1β* mRNA expression: age, sex, percentage body fat, birth-weight, zygosity, the *PGC-1β* Ala203Pro polymorphism and the interaction between age and the *PGC-1β* Ala203Pro polymorphism. When correcting for these variables in a regression analysis, only age was significantly related to skeletal muscle *PGC-1β* expression (basal expression, regression coefficient = -0.01 ; $p=0.003$; insulin-stimulated expression, regression coefficient = -0.02 ; $p<0.0005$).

Impact of the *PGC-1β* Ala203Pro polymorphism on in vivo glucose and fat metabolism Young carriers of a *PGC-1β* 203Pro allele had increased glucose storage or non-oxidative glucose metabolism (NOGM; 48.8 ± 5.4 vs 38.0 ± 1.6 $\mu\text{mol kg LBM}^{-1} \text{ min}^{-1}$ [8.78 ± 0.97 vs 6.83 ± 0.28 $\text{mg kg LBM}^{-1} \text{ min}^{-1}$]; $p=0.025$) and glycolytic flux (GF; 30.9 ± 3.2 vs 24.1 ± 1.0 $\mu\text{mol kg LBM}^{-1} \text{ min}^{-1}$ [5.56 ± 0.57 vs 4.34 ± 0.19 $\text{mg kg LBM}^{-1} \text{ min}^{-1}$]; $p=0.026$) during insulin stimulation compared with the Ala/Ala group (Table 1). However, no significant influence of this polymorphism on glucose disposal, glucose and fat oxidation was detected in the young twins (Table 1). Furthermore, this polymorphism did not influence glucose or fat metabolism in the elderly twins (data not shown).

We then performed a regression analysis to test whether any of the following variables influenced insulin-stimulated glucose disposal and storage, glucose and fat oxidation, and GF: sex, percentage body fat, birth-weight, zygosity, the *PGC-1β* Ala203Pro polymorphism and both basal and insulin-stimulated muscle *PGC-1β* mRNA expression

Table 1 The influence of the *PGC-1β* Ala203Pro polymorphism on insulin-stimulated metabolic variables in young twins

Variable	Ala/Ala	Ala/Pro+Pro/Pro	<i>p</i> value
	<i>n</i> =94	<i>n</i> =12	
Glucose disposal rate			
$\mu\text{mol kg LBM}^{-1} \text{ min}^{-1}$	62.6 ± 2.0	73.6 ± 5.3	0.09
$\text{mg kg LBM}^{-1} \text{ min}^{-1}$	11.52 ± 0.33	13.26 ± 0.96	
NOGM			
$\mu\text{mol kg LBM}^{-1} \text{ min}^{-1}$	38.0 ± 1.6	48.8 ± 5.4	0.025
$\text{mg kg LBM}^{-1} \text{ min}^{-1}$	6.83 ± 0.28	8.78 ± 0.97	
Glucose oxidation			
$\mu\text{mol kg LBM}^{-1} \text{ min}^{-1}$	26 ± 0.81	24.8 ± 1.5	0.8
$\text{mg kg LBM}^{-1} \text{ min}^{-1}$	4.68 ± 0.15	4.47 ± 0.27	
GF			
$\mu\text{mol kg LBM}^{-1} \text{ min}^{-1}$	24.1 ± 1.0	30.9 ± 3.2	0.026
$\text{mg kg LBM}^{-1} \text{ min}^{-1}$	4.34 ± 0.19	5.56 ± 0.57	
Fat oxidation ($\text{mg kg LBM}^{-1} \text{ min}^{-1}$)	0.33 ± 0.05	0.38 ± 0.09	0.76

Data are expressed as means±SEM

To adjust for the lack of independence between MZ and DZ twins, all comparisons of mean differences between groups were performed using generalised estimation equations (GEE) methodology

(Table 2). The regression analysis was performed separately in young and elderly twins, since the analysis above (Table 1) only demonstrated effects of the *PGC-1 β* Ala203Pro polymorphism in young twins. After correction for covariates, the *PGC-1 β* 203Pro allele was consistently positively related to insulin-stimulated NOGM ($p=0.03$) and GF ($p=0.001$) in the young twins (Table 2). Furthermore, in agreement with our previous study [5], basal *PGC-1 β* mRNA expression was positively related to NOGM ($p=0.005$) and fat oxidation ($p=0.006$) and negatively related to glucose oxidation ($p=0.02$; Table 2). Finally, when performing regression analysis in the elderly twins, the *PGC-1 β* Ala203Pro polymorphism was found to be not significantly related to any of the metabolic variables studied (data not shown).

PGC-1 β mRNA expression correlates with markers for oxidative fibre types in human skeletal muscle We investigated whether basal *PGC-1 β* expression correlates with basal mRNA expression of markers for three different fibre types, namely *MHC7* (type I fibres, slow-twitch oxidative), *MHCIIa* (type IIA fibres, fast-twitch oxidative) and *MHCIIx/d* (type IIB fibres, fast-twitch glycolytic), in human skeletal muscle ($n=156$, young and elderly twins).

Table 2 Factors influencing insulin-stimulated glucose disposal rate, glucose storage (NOGM), glucose oxidation, glycolytic flux and fat oxidation in skeletal muscle of 110 young twins

Variable	Regression coefficient	<i>p</i> value
Glucose disposal rate		
Sex (male vs female)	2.4	0.02
Percentage body fat	−0.17	0.01
Zygoty (MZ vs DZ)	−2.1	0.007
NOGM		
Percentage body fat	−0.09	0.025
Zygoty (MZ vs DZ)	−1.8	0.003
<i>PGC-1β</i> Ala203Pro polymorphism	2.2	0.03
<i>PGC-1β</i> gene expression, basal	0.83	0.005
Glucose oxidation		
Sex (male vs female)	1.1	0.0001
<i>PGC-1β</i> gene expression, basal	−0.35	0.02
GF		
<i>PGC-1β</i> Ala203Pro polymorphism	2.1	0.001
Fat oxidation		
<i>PGC-1β</i> gene expression, basal	0.15	0.006

By regression analysis we tested whether the following factors influence insulin-stimulated glucose disposal, glucose storage (NOGM), glucose oxidation, glycolytic flux and fat oxidation: sex (men coded 0, women coded 1), percentage body fat, birth-weight, zygoty, the *PGC-1 β* Ala203Pro polymorphism and both basal and insulin-stimulated skeletal muscle *PGC-1 β* gene expression $p<0.05$ for all final models

While both *MHC7* and *MHCIIa* correlated positively with *PGC-1 β* expression ($r=0.28$; $p=0.0005$ and $r=0.31$; $p=0.00008$, respectively), there was no correlation between *MHCIIx/d* and *PGC-1 β* ($r=-0.03$; $p=0.6$). Moreover, elderly twins had 25% lower mRNA expression of *MHCIIa* than young twins ($p=0.0005$). However, the mRNA expression of *MHC7* and *MHCIIx/d* did not change with age ($p=0.5$ and $p=0.9$, respectively). Finally, because *PGC-1 β* correlated with *MHC7* and *MHCIIa* expression, we tested whether the *PGC-1 β* Ala203Pro polymorphism influenced the basal expression of these two fibre type markers in young and elderly twins. Indeed, elderly carriers of a *PGC-1 β* 203Pro allele ($n=18$) had increased *MHC7* expression (2.1 ± 0.3 vs 1.6 ± 0.1 ; $p<0.05$) compared with the Ala/Ala group ($n=62$). However, there was no significant influence of this polymorphism on basal *MHCIIa* expression in elderly twins or on *MHC7* and *MHCIIa* expression in young twins (data not shown).

Discussion

PGC-1 β expression is reduced in muscle of healthy elderly individuals and in patients with type 2 diabetes mellitus [3, 5]. In addition, *PGC-1 β* was previously found to increase the expression of mitochondrial genes and the formation of oxidative fibres in rodent muscle [1, 7–9]. We have also shown that this co-activator is positively related to NOGM and lipid oxidation in human muscle in vivo [5]. In the present study we found that in vivo measures of NOGM and GF were significantly increased in young carriers of the *PGC-1 β* 203Pro allele. Our data also suggest that *PGC-1 β* influences the formation of oxidative fibres in human skeletal muscle.

The risk of developing obesity and metabolic disease including insulin resistance, dyslipidaemia and type 2 diabetes mellitus increases with high-fat/high-energy diets and reduced physical activity. However, there is a large inter-individual variation in the metabolic response to diet and exercise and not all individuals exposed to a sedentary lifestyle and poor diets actually develop obesity or metabolic disease. A likely reason is that inherited factors influence the susceptibility to obesity; indeed, we recently identified such a genetic factor, the common *PGC-1 β* Ala203 allele, which we found to be associated with increased risk of obesity [6]. The present study demonstrates further how this polymorphism influences glucose metabolism in an age-dependent manner. *PGC-1 β* was previously suggested to influence metabolism in rodents by co-activating the ERR, resulting in elevated energy expenditure and resistance to obesity [12]. The *PGC-1 β* Ala203Pro polymorphism changes an amino acid from alanine to proline, which might influence the ability of

PGC-1 β to co-activate transcription factors, e.g. ERR. This may explain, or at least contribute to, the positive effects of the *PGC-1 β* 203Pro allele on peripheral (i.e. muscle) glucose metabolism in the young. The fact that elderly 203Pro carriers did not show an age-related reduction in muscle *PGC-1 β* expression could be interpreted as an attempt to protect these subjects against reductions in glucose metabolism as they age. Despite this, we found no positive influence of the *PGC-1 β* 203Pro allele on glucose metabolism in the elderly, suggesting that these subjects cannot compensate for the impact of other degenerative factors influencing the decline in metabolism during ageing.

In the present study we were unable to identify an influence of the *PGC-1 β* Ala203Pro polymorphism on measures of obesity such as percentage body fat, BMI and WHR (data not shown). The most plausible reason for this is lack of statistical power, as this study included 196 twins compared with 7,790 middle-aged subjects in our previous case–control study [6].

Our detailed metabolic studies allowed us to gain insight into distinct effects of the *PGC-1 β* Ala203Pro polymorphism on in vivo glucose metabolism and cellular glucose partitioning. Indeed, this polymorphism influenced NOGM (glycogen synthesis) as well as ‘exogenous’ (i.e. extracellular-derived glucose uptake) GF rates, as determined by the appearance rate of ^3H -labelled water. This is in contrast to the glucose oxidation rate as determined from indirect calorimetry, which measures the oxidation of glucose from both intra- and extracellular components. We have previously reported apparently divergent results regarding glucose oxidation and GF rates [13], which are likely to be explained in particular by the unknown contribution of glucose oxidation from endogenous glycogen pools. In this study, the *PGC-1 β* Ala203Pro polymorphism influenced insulin-stimulated GF but not glucose oxidation in the young twins. The explanation for this may be that the influence is exerted at the level of membrane glucose transport and that the intracellular response to that may be a compensatory elevated rate of glycogenolysis, resulting in near-normal total (endogenous and exogenous) glucose oxidation.

Recent studies of over-production of *PGC-1 β* in rodent muscle suggest that *PGC-1 β* plays a positive role in the formation of oxidative fibres in skeletal muscle [7, 8]. Moreover, soleus muscle from mice lacking *PGC-1 β* showed impaired ATP-production and reduced expression of genes from the respiratory chain [9]. The present study suggests that *PGC-1 β* also influences the formation of oxidative fibres in human muscle, since the expression of *PGC-1 β* correlated positively with markers for both slow- and fast-twitch oxidative fibres. In contrast, Krämer et al. [14] did not find any correlation between the expression

level of *PGC-1 β* and fibre type composition in human muscle, which could be explained by the small number of subjects ($n=20$).

In summary, our data provide evidence that carriers of a *PGC-1 β* 203Pro allele have enhanced rates of insulin-stimulated glucose metabolism when young and may be protected against an age-related decline in skeletal muscle gene expression of this key co-activator of energy metabolism. This study also proposes a role for *PGC-1 β* in determining fibre type composition in human skeletal muscle.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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