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ARTICLE

Epigenetic regulation of *PPARGC1A* in human type 2 diabetic islets and effect on insulin secretion

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Abstract
Aims/hypothesis Insulin secretion in pancreatic islets is dependent upon mitochondrial function and production of ATP. The transcriptional coactivator peroxisome proliferator activated receptor gamma coactivator-1 alpha (protein PGC-1α; gene *PPARGC1A*) is a master regulator of mitochondrial genes and its expression is decreased and related to impaired oxidative phosphorylation in muscle from patients with type 2 diabetes. Whether it plays a similar role in human pancreatic islets is not known. We therefore investigated if *PPARGC1A* expression is altered in islets from patients with type 2 diabetes and whether this expression is influenced by genetic (*PPARGC1A* Gly482Ser polymorphism) and epigenetic (DNA methylation) factors. We also tested if experimental downregulation of *PPARGC1A* expression in human islets influenced insulin secretion.
Methods The *PPARGC1A* Gly482Ser polymorphism was genotyped in human pancreatic islets from 48 non-diabetic and 12 type 2 diabetic multi-organ donors and related to *PPARGC1A* mRNA expression. DNA methylation of the *PPARGC1A* promoter was analysed in pancreatic islets from ten type 2 diabetic and nine control donors. Isolated human islets were transfected with *PPARGC1A* silencing RNA (siRNA).
Results *PPARGC1A* mRNA expression was reduced by 90% ($p<0.005$) and correlated with the reduction in insulin secretion in islets from patients with type 2 diabetes. After downregulation of *PPARGC1A* expression in human islets by siRNA, insulin secretion was reduced by 41% ($p\leq 0.01$). We were able to ascribe reduced *PPARGC1A* expression in islets to both genetic and epigenetic factors, i.e. a common *PPARGC1A* Gly482Ser polymorphism was associated with reduced *PPARGC1A* mRNA expression ($p<0.00005$) and reduced insulin secretion ($p<0.05$). In support of an epigenetic influence, the *PPARGC1A* gene promoter showed a twofold increase in DNA methylation in diabetic islets compared with non-diabetic islets ($p<0.04$).
Conclusions/interpretation We have shown for the first time that *PPARGC1A* might be important in human islet insulin secretion and that expression of *PPARGC1A* in human islets can be regulated by both genetic and epigenetic factors.

Keywords DNA methylation · Epigenetic · Gene expression · Genetic · Human · Pancreatic islets · PGC-1α · *PPARGC1A* · Type 2 diabetes

Abbreviations

HNF-1	hepatic nuclear factor 1
NA	nicotinamide
PGC-1α	peroxisome proliferator activated receptor gamma coactivator-1 alpha
siRNA	silencing RNA
STZ	streptozotocin

Introduction

Type 2 diabetes is characterised by chronic hyperglycaemia as a result of impaired pancreatic beta cell function and

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insulin resistance in peripheral tissues, i.e. skeletal muscle, adipose tissue and liver. Although each of these pathogenic defects could be accounted for by specific mechanisms, impaired ATP production as a consequence of reduced oxidative phosphorylation might provide an intriguing common pathogenic pathway for all these defects. The transcriptional coactivator peroxisome proliferator activated receptor gamma coactivator-1 alpha (protein PGC-1α; gene *PPARGC1A*) is an important factor regulating the expression of genes for oxidative phosphorylation and ATP production in target tissues through coactivation of nuclear receptors [1]. We have previously shown that the expression of *PPARGC1A* and a set of genes involved in oxidative phosphorylation is reduced in skeletal muscle from patients with type 2 diabetes [2]. Furthermore, a common polymorphism, Gly482Ser, in the *PPARGC1A* gene has been associated with increased risk of type 2 diabetes and an age-related reduction in muscle *PPARGC1A* expression [3–5]. In addition, genetic variation in the *PPARGC1A* gene was associated with indices of beta cell function [6]. Despite the central role of ATP for insulin secretion, the function of PGC-1α in human pancreatic islets and beta cells is less well established [7].

Obesity, reduced physical activity and ageing are well known risk factors for type 2 diabetes. However, all individuals exposed to an affluent environment do not develop the disease. One likely reason is that genetic variation modifies individual susceptibility to the environment. However, the environment could also modify genetic risk factors by influencing expression of a gene by DNA methylation or histone modifications. Cytosine residues occurring in CG dinucleotides are targets for DNA methylation and gene expression is usually reduced when DNA methylation takes place at a promoter. Whether DNA methylation influences gene expression in target tissues for type 2 diabetes and thereby the pathogenesis of the disease remains to be demonstrated.

The present study investigated: (1) whether expression of *PPARGC1A* is altered in human islets from patients with type 2 diabetes; (2) if this expression is influenced by genetic (the *PPARGC1A* Gly482Ser polymorphism) and epigenetic (DNA methylation) factors; and (3) if expression of *PPARGC1A* influences insulin secretion.

Methods

Multi-organ donors The characteristics of the 48 non-diabetic and 12 type 2 diabetic multi-organ donors, whose pancreases were processed for islet preparation, are presented in Table 1. Pancreases were obtained and processed with the approval of the regional Ethics Committee.

Table 1 Clinical characteristics of type 2 diabetic and non-diabetic donors

	Non-diabetic donors	Type 2 diabetic donors	*p* value
n (male/female)	48 (30/18)	12 (6/6)	
	9 (7/2)[a]	10 (5/5)[a]	
Age (years)	53.2±2.4	66.7±2.4	<0.05
	54.2±3.5[a]	65.1±2.6[a]	
BMI (kg/m^2)	24.8±0.6	27.1±1.0	<0.05
	25.9±1.3[a]	26.9±1.1[a]	
Gly/Gly (%)[b]	53.3	27.3	
Gly/Ser+Ser/Ser (%)[b]	46.7	72.3	

Data are expressed as mean±SEM
[a] Donors used for DNA methylation analysis
[b] *PPARGC1A* Gly482Ser polymorphism

Human pancreatic islets and experimental plan Isolated pancreatic islets were prepared by collagenase digestion and density gradient purification [8, 9]. After isolation, islets were cultured free floating in M199 culture medium (Sigma-Aldrich, St Louis, MO, USA) at 5.5 mmol/l glucose concentration and studied within 3 days from isolation. Cell viability, measured by Trypan Blue exclusion, was higher than 90% in control and diabetic islets after 3 days in culture.

Insulin secretion study Insulin secretion studies were performed as previously described [8, 9]. Following a 45 min pre-incubation period at 3.3 mmol/l glucose, groups of 30 islets of comparable size were kept at 37°C for 45 min in KRB, 0.5% (wt/wt) albumin, pH 7.4, containing 3.3 mmol/l glucose. At the end of this period, the medium was completely removed and replaced with KRB containing either 3.3, 16.7 or 3.3 mmol/l glucose plus 20 mmol/l arginine, or 3.3 mmol/l glucose plus 100 μmol/l glibenclamide. After an additional 45 min incubation period, the medium was removed. Media (500 μl aliquots from the 10 ml incubation volume) were stored at −20°C until insulin concentrations were measured by immunoradiometric assay (Pantec Forniture Biomediche, Turin, Italy).

Animals Streptozotocin (STZ)–nicotinamide (NA)-treated male Wistar rats (2–3 months old) were administered 210 mg/kg NA i.p. (Sigma, St Louis, MO, USA) dissolved in saline, 15 min before an i.v. injection of 60 mg/kg STZ (Sigma) that had been dissolved in citrate buffer (pH 4.5) immediately before use. Control rats were injected with vehicle alone. STZ–NA-treated animals had stable hyperglycaemia (8.9–10.0 mmol/l) and they were used for the experiments 5 weeks after diabetes was induced. Pancreatic islets were isolated by the collagenase method using the procedure of pancreatic duct cannulation and density gradient purification as described elsewhere [10, 11].

GK rats were obtained from the Stockholm colony and bred as described [12]. Inbred, normoglycaemic F344 rats were purchased from Charles River Laboratories (Wilmington, MA, USA) and maintained by sister–brother mating. Transfer of GK alleles onto the genome of F344 rats by repeated backcrossing (ten generations) established the homozygous congenic strains F344.GK-*Niddm1f* (NIDD M1F) and F344.GK-*Niddm1i* (NIDDM1I). NIDDM1F carries 0.5% of the GK genotype (8 cM), based on genetic distance, on a homozygous F344 genetic background. NIDDM1F rats display hyperglycaemia accompanied by fasting hyperinsulinaemia and increased epididymal fat, implicating insulin resistance. NIDDM1I carries 0.8% of the GK genotype (14 cM), and display hyperglycaemia and insulin secretion defects [13–15]. Backcrossing was designed to introduce mitochondrial DNA and chromosomes X plus Y from F344. The congenic strains were kept constant by sister–brother mating for several generations. To avoid effects of the oestrous cycle and other sex-specific influences, only male rats were included in this study. Rats were maintained at constant temperature and humidity in a 12 h light–dark cycle with free access to standard laboratory chow pellets and water. All experiments were approved by the local Ethics Committees. Isolated pancreatic islets were prepared from rats at 8 weeks of age after a 6 h fast (08:00–14:00 hours), by injection of a collagenase solution via the bile–pancreatic duct [16].

Analysis of PPARGC1A *mRNA expression in pancreatic islets* Total RNA was extracted from human islets, after 3 days in culture, using the RNeasy Protect Mini Kit (Qiagen, Valencia, CA, USA). It was quantified by absorbance at A_{260}/A_{280} nm (ratio>1.65) in a Perkin-Elmer spectrophotometer (Waltham, MA, USA) and its integrity was assessed after electrophoresis in 1.0% (wt/wt) agarose gels by ethidium bromide staining. Human and rat *PPARGC1A* mRNA expression were quantified by RT-PCR [8]. Gene-specific probes and primer pairs for *PPARGC1A* (Assays-on-demands, human, Hs00173304_m1 and rat, Rn00580241_A1; Applied Biosystems, Foster City, CA, USA) were used. Each sample was run in duplicate and the transcript quantity was normalised to the mRNA level of cyclophilin A (human, 4326316E and rat Rn00574762_A1; Applied Biosystems). For each probe/primer set, a standard curve was generated, which was confirmed to increase linearly with increasing amounts of cDNA.

Downregulation of PGC-1α in human islets using silencing RNA (siRNA) In order to test whether inhibition of *PPARGC1A* expression could directly modulate insulin release, isolated human islets were transfected with *PPARGC1A* siRNA by using Arrest-In Transfection (Open Biosystem; Celbio, Pero, Italy). This is a polymeric formulation developed and optimised for highly efficient delivery of siRNA into the nucleus of suspension cells in the presence of serum-containing medium. Pre-designed Silencer siRNAs and Silencer non-targeting siRNAs (negative control) for *PPARGC1A* (Ambion, Austin, TX, USA) were used. Transfection was performed according to the manufacturer's instructions. Briefly, islets obtained from five pancreases were washed 12 times in KRB and exposed for 10 min to free Ca^{2+}- and Mg^{2+}-KRB to allow cell disaggregation. At the end of the incubation period, 400 islets per study point were re-suspended in 800 μl M199 medium (Sigma-Aldrich) added with adult bovine serum. siRNA (80 nmol/l) was diluted in 100 μl M199, while 20 μg Arrest-In solution (Celbio) was dissolved in 100 μl M199 and incubated for 10 min after rapid mixing to allow formation of transfection complexes. Finally, 200 μl of this solution were added into wells containing islets and incubation was allowed for 48 h in a CO_2 incubator at 37°C. At the end of the incubation period well volume was doubled with M199 culture medium and samples were kept in the incubator for another 48 h, when islets function, viability, transfection efficiency (60% when measured by Polyfectamine tied to a fluorescent probe) and gene expression were evaluated.

Genotyping Genomic DNA was extracted from pancreatic islets using a Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). The Gly482Ser (GGT→AGT) polymorphism of *PPARGC1A* was genotyped using an allelic discrimination assay performed with an ABI 7900 system (Applied Biosystems), using PCR primers: 5′-CACTTCGG TCATCCCAGTCAA-3′ (forward) and 5′-TTATCACTTT CATCTTCGCTGTCATC-3′ (reverse), and TaqMan MGB probes: Fam-5′-AGACAAGACCGGTGAA-3′ and Vic-5′-CAGACAAGACCAGTGAA-3′ [5, 17].

DNA methylation A sequence starting 5,000 bp upstream from the *PPARGC1A* translation start was used in MethPrimer (http://www.urogene.org/methprimer/index.html) to search for regions with CpG sites and PCR designs. A *PPARGC1A* sequence 986–746 bases upstream from the translation start including four possible DNA-methylation sites and a putative hepatic nuclear factor 1 (HNF-1) binding site, was selected and analysed for DNA methylation (Fig. 1c). Genomic DNA, isolated from pancreatic islets of nine non-diabetic and ten type 2 diabetic multi-organ donors, was treated with bisulphite using the EZ DNA Methylation Kit (Zymo Research, Orange, CA, USA). Bisulphite-modified DNA was amplified by nested PCR with primers designed using MethPrimer. Primer pair 1: 5′-TAGGGTATTAGGG TTGGAATTTAATG-3′ (forward) and 5′-CCCATAACAA TAAAAAATACCAACTC-3′ (reverse), and primer pair 2 (used for nested PCR): 5′-TATTTTAAGGTAGTTAGG

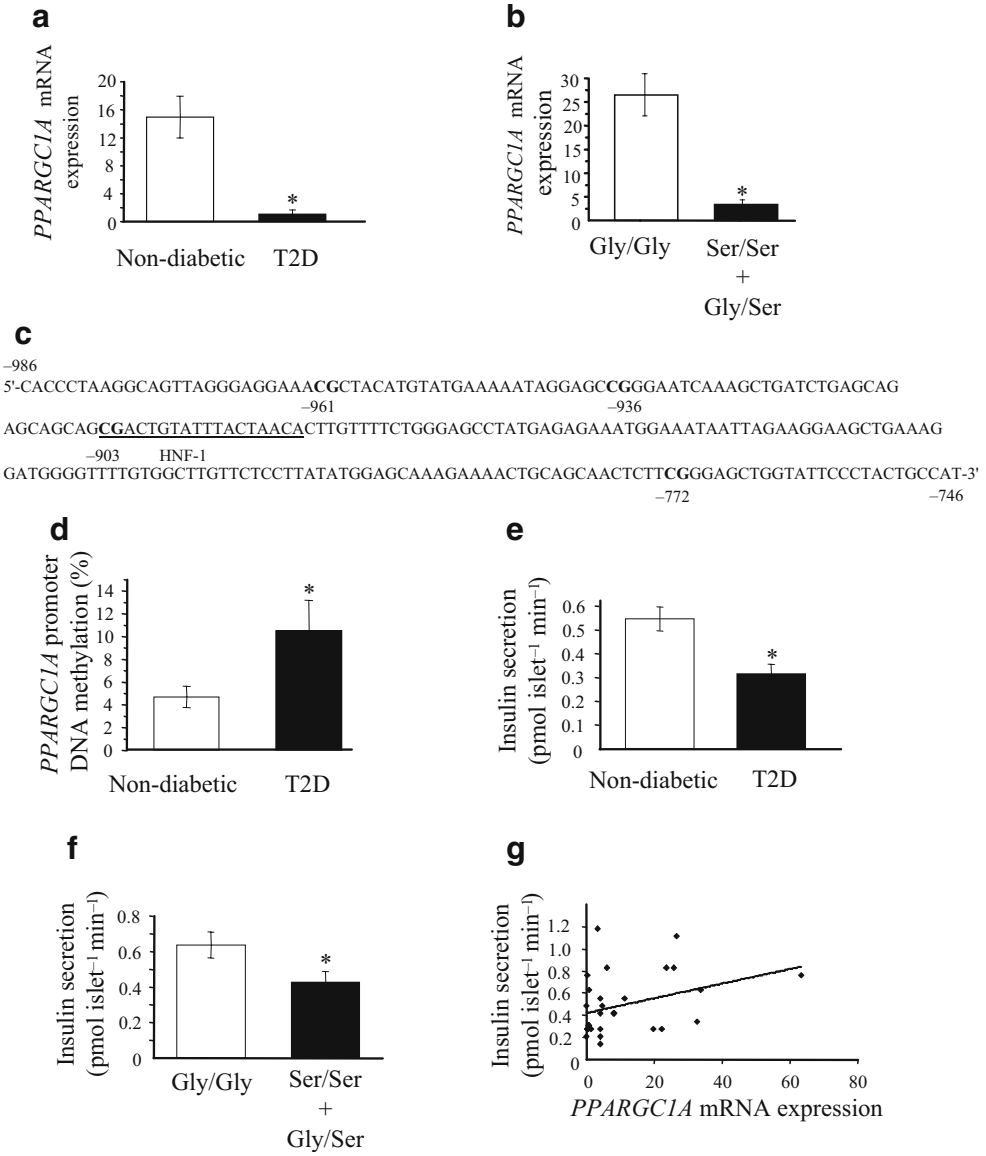

Fig. 1 *PPARGC1A* mRNA expression in human pancreatic islets is influenced by type 2 diabetes, a *PPARGC1A* Gly482Ser polymorphism and DNA methylation. The influence of **a** type 2 diabetes and **b** the *PPARGC1A* Gly482Ser polymorphism on *PPARGC1A* mRNA expression in human pancreatic islets. **c** The *PPARGC1A* promoter sequence investigated, showing the four DNA methylation target sites; −772, −903, −936 and −961 and a putative binding-site for HNF-1. **d** The influence of type 2 diabetes on DNA methylation of the *PPARGC1A* promoter. The influence of **e** type 2 diabetes (T2D) and **f** the *PPARGC1A* Gly482Ser polymorphism on absolute insulin release (pmol islet^{-1} min^{-1}) in response to 16.7 mmol/l glucose. **g** Correlations between *PPARGC1A* mRNA expression and absolute insulin release (pmol islet^{-1} min^{-1}) in response to 16.7 mmol/l glucose in human pancreatic islets (r=0.38, p<0.05). Results are expressed as mean±SEM. *p<0.05

GAGGAAA-3′ (forward) and 5′-ATAACAATAAAAAA TACCAACTCCC-3′ (reverse). The PCR products were then cloned into a vector (TOPO TA Cloning Kit for Sequencing, Invitrogen, Carlsbad, CA, USA) and ten colonies from each donor were purified with a Miniprep kit (Qiagen). These individual clones were sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The number of methylated sites was determined and divided by the total number of methylation sites and then multiplied by 100 to show the percentage of methylation for each donor.

Statistical methods Differences in *PPARGC1A* mRNA expression, percentage of DNA methylation and insulin secretion between the different groups studied were analysed using Student's t test or the non-parametric Mann–Whitney test, where appropriate. Correlations were calculated using Pearson correlation coefficients for normally distributed values and Spearman correlation coefficients when normality was rejected. Log values were used in the multivariate regression analysis. Differences in expression between GK, F344, NIDDM1F and NIDDM1I rats were analysed using one-way ANOVA, followed by a Kruskal–Wallis Z test. All p values were two-tailed and p values less than 0.05 were considered significant. Statistical calculations were performed by NCSS software (NCSS Statistical Software, Kaysville, UT, USA).

Results

The mRNA expression of *PPARGC1A* was markedly reduced in pancreatic islets from type 2 diabetic compared

with non-diabetic donors (1.06±0.63 vs 15.0±3.0; $p<0.005$; Fig. 1a). Intriguingly, non-diabetic carriers of a *PPARGC1A* 482Ser allele, Gly/Ser or Ser/Ser genotype carriers had markedly lower *PPARGC1A* mRNA expression compared with carriers of the Gly/Gly genotype [Gly/Ser + Ser/Ser 3.43±0.94 ($n=17$) vs Gly/Gly 26.53±4.42 ($n=17$); $p<0.00005$] (Fig. 1b). However, there was no difference in *PPARGC1A* mRNA expression between non-diabetic carriers of the Gly/Ser (3.9±4.0; $n=11$) or Ser/Ser (2.7±5.5; $n=6$) genotypes. This demonstrates that the effect of the genotype on gene expression is seen already in the pre-diabetic state.

The diabetic donors were significantly older and had higher BMI than control donors (Table 1). We therefore tested whether these factors might also relate to *PPARGC1A* expression in human islets using a multivariate regression analysis including age, BMI and disease status as covariates. Only disease status was significantly associated with *PPARGC1A* expression in this analysis ($r=1.04$; $p=0.0065$).

We next evaluated whether epigenetic phenomena such as DNA methylation would influence *PPARGC1A* expression in islets. Cytosine residues occurring in CG dinucleotides are targets for DNA methylation, and gene expression is usually reduced when DNA is methylated at the promoter. We used bisulphite sequencing to assess DNA methylation of four methylation target sites, −772, −903, −936 and −961 in the *PPARGC1A* promoter in pancreatic islets from the type 2 diabetic and non-diabetic human multi-organ donors (Fig. 1c). Interestingly, there was an approximately twofold increase in DNA methylation of the *PPARGC1A* promoter of diabetic compared with non-diabetic human islets (10.5±2.7 vs 4.7±0.9%; $p<0.04$; Fig. 1d). Also in this subset of donors, the *PPARGC1A* mRNA expression was significantly reduced in diabetic compared with non-diabetic islets ($p=0.002$). There was a trend towards an inverse correlation between the level of DNA methylation and *PPARGC1A* mRNA expression ($r=-0.48$; $p=0.08$, after adjustment for disease status).

Insulin release (pmol islet^{-1} min^{-1}) in response to 16.7 mmol/l glucose was reduced in type 2 diabetic compared with non-diabetic islets (0.32±0.04 vs 0.55±0.05; $p<0.01$) as well as in non-diabetic islets carrying the *PPARGC1A* 482Ser allele (Gly/Ser+Ser/Ser) compared with carriers of the Gly/Gly genotype (Gly/Ser+Ser/Ser 0.43±0.06 vs Gly/Gly 0.64±0.07; $p<0.05$; Fig. 1e–f). In addition, the mRNA expression of *PPARGC1A* correlated positively with glucose-mediated insulin release ($r=0.38$; $p<0.05$; Fig. 1g). Moreover, the *PPARGC1A* Gly482Ser polymorphism did not significantly affect basal insulin secretion at 3.3 mmol/l glucose (Gly/Ser+Ser/Ser, 0.23±0.01 vs Gly/Gly, 0.29±0.02; $p=0.13$), or insulin response to arginine (Gly/Ser+Ser/Ser 0.48±0.05 vs Gly/Gly 0.53±0.08; $p=0.77$) or glibenclamide (Gly/Ser+Ser/Ser 0.59±0.1 vs Gly/Gly 0.56±0.09; $p=0.98$).

Having seen the relationship between *PPARGC1A* expression and insulin secretion, we set out to determine whether modulation of *PPARGC1A* expression would influence insulin secretion. This was achieved by studying insulin secretion after experimental downregulation of PGC-1α in human islets transfected with *PPARGC1A* siRNA ($n=3$–5 experiments). As shown in Fig. 2, transfection with siRNA was associated with a 45% reduction in the mRNA expression of *PPARGC1A* ($p=0.01$), 32% reduction in insulin mRNA ($p=0.05$) and 41% reduction of the insulin stimulation index (i.e. incremental folds above baseline insulin release; $p=0.01$). Notably, no significant change was seen in glucagon mRNA expression, suggesting that the effect was primarily due to a downregulation of PGC-1α in beta cells. As a negative control, transfection with scrambled siRNA was not associated with any changes in *PPARGC1A* (1.26±0.25), insulin (1.29±0.16) or glucagon (0.99±0.15) mRNA expression ($p>0.05$).

The finding of reduced *PPARGC1A* expression in human diabetic islets was somewhat surprising given an earlier report of increased PGC-1α levels in islets from diabetic mice (*ob*/*ob*) and rat (ZDF and pancreatectomised rats) models [7]. To further explore species-specific differences we also measured *PPARGC1A* mRNA expression in pancreatic islets from two diabetic rat models. *PPARGC1A* expression was reduced in islets from STZ–NA diabetic male Wistar rats compared with control animals (3.08±1.07 vs 10.26±1.12; $p<0.009$; Fig. 3a). Moreover, *PPARGC1A* expression was reduced in islets from the congenic NIDDM1I strain, which has insulin secretion defects, compared with the insulin-resistant NIDDM1F strain (7.18±1.79 vs 11.30±1.79; $p<0.02$) of the GK rat, an animal model of polygenic type 2 diabetes (Fig. 3b). However, no difference in *PPARGC1A* expression was found when comparing islets from F344 or GK rat strains (Fig. 3b).

Discussion

The key results from the present study were that *PPARGC1A* mRNA expression and insulin secretion were reduced in pancreatic islets of patients with type 2 diabetes and in non-diabetic carriers of a *PPARGC1A* 482Ser allele, a genotype previously associated with the disease. Moreover, we demonstrate that epigenetic mechanisms are likely to be operative in the pathogenesis of type 2 diabetes since DNA methylation of the *PPARGC1A* promoter was increased in human diabetic islets. Experimental downregulation of *PPARGC1A* expression in human islets by siRNA resulted in decreased insulin secretion thereby demonstrating a causal link between PGC-1α levels and insulin secretion. Based on these observations we propose a model where combinations of genetic and epigenetic factors can influ-

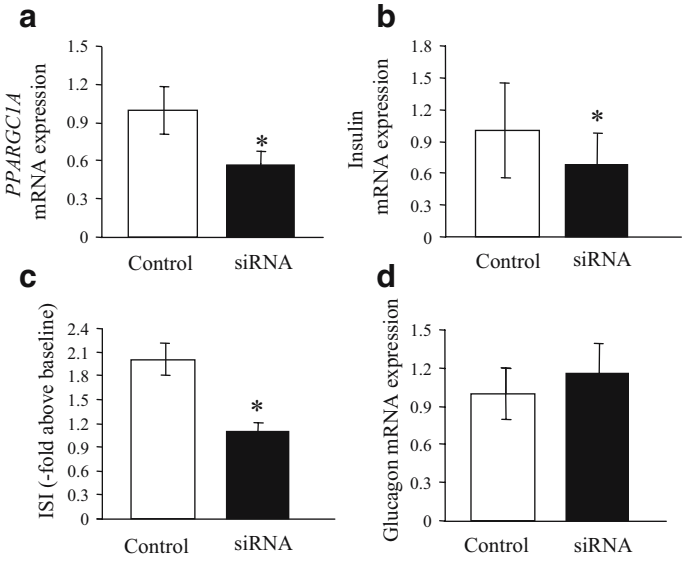

Fig. 2 Transfection of human pancreatic islets with *PPARGC1A* siRNA is associated with reduced mRNA levels of **a** *PPARGC1A* (n=3) and **b** insulin (n=5) and **c** concomitant reduction of the insulin stimulation index (ISI), i.e. incremental fold change above basal insulin release. **d** Conversely, inhibition of *PPARGC1A* expression in human pancreatic islets has no effect on glucagon mRNA expression (n=5). Results are expressed as mean±SEM. *p<0.05

ence the level of PGC-1α in human islets and subsequently glucose-stimulated insulin secretion.

The transcriptional coactivator PGC-1α is an important factor regulating the expression of genes for oxidative phosphorylation in a number of tissues including skeletal muscle, liver and adipose tissue. We and others have previously demonstrated reduced expression of *PPARGC1A* and a set of genes involved in oxidative phosphorylation in skeletal muscle from patients with type 2 diabetes [2, 18]. However, the level and role of PGC-1α in pancreatic islets of patients with type 2 diabetes remained to be determined. The present results indicate that the situation in human islets mirrors the situation in skeletal muscle, since the expression level of *PPARGC1A* was reduced in human type 2 diabetic islets, thus providing evidence for a common defect that may simultaneously contribute to peripheral insulin resistance and impairment of beta cell function.

In contrast to the present results in humans, elevated PGC-1α levels have been reported in islets from diabetic rodent models, *ob*/*ob* mice, ZDF rats and pancreatectomised rats [7]. In these rodent models, the increase in PGC-1α was associated with suppressed beta cell metabolism and insulin release. This could reflect true species differences, but also differences in experimental conditions. To address this issue, we determined: (1) the effect of inhibiting PGC-1α production by transfecting human pancreatic islets with *PPARGC1A* siRNA; and (2) *PPARGC1A* expression in pancreatic islets of different rodent models with experimental diabetes, i.e. STZ-diabetic Wistar rats and congenic strains from the type 2 diabetes-like GK rat. Silencing PGC-1α production was associated with concomitant reduction of insulin mRNA expression and insulin release in response to glucose. This effect appears to be highly specific for insulin regulation as it had no effect

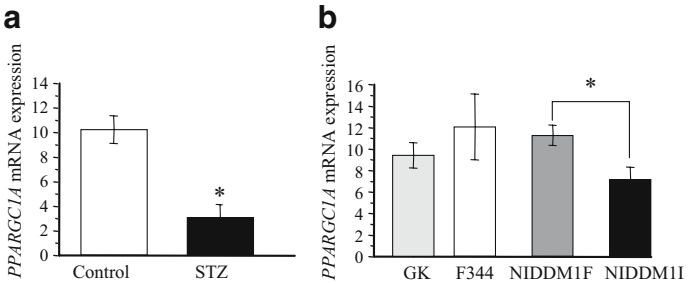

Fig. 3 *PPARGC1A* mRNA expression in rodent pancreatic islets. **a** Male Wistar rats (2–3 months old) were treated with STZ–NA (n=5) or vehicles (control; n=5) [10, 11] and *PPARGC1A* mRNA expression together with the internal standard cyclophilin A was analysed in pancreatic islets isolated from STZ–NA-treated animals showing a stable hyperglycaemia (8.9–10.0 mmol glucose/l) and controls. **b** *PPARGC1A* mRNA expression together with the internal standard cyclophilin A was analysed in pancreatic islets prepared from rats at 8 weeks of age after a 6 h fast. GK rats (n=6) were obtained from the Stockholm colony and bred as described [12]. Inbred, normoglycaemic F344 (n=6) were purchased from Charles River Laboratories. Transfer of GK alleles onto the genome of F344 rats by repeated backcrossing (ten generations) established the homozygous congenic strains NIDDM1F and NIDDM1I. NIDDM1F rats (n=6) carry 0.5% of the GK genotype, based on genetic distance, on a homozygous F344 genetic background (8 cM) and display hyperglycaemia accompanied by fasting hyperinsulinaemia, implicating insulin resistance. NIDDM1I (n=6) carries 0.8% of the GK genotype (14 cM) and display insulin secretion defects [13–15]. Results are expressed as mean±SEM. *p<0.05

whatsoever on glucagon mRNA expression. The congenic strains have been bred from the GK rat based upon low insulin secretion or insulin resistance. In line with the results observed in human islets, *PPARGC1A* expression was reduced in islets from diabetic animals and in rat strains with suppressed insulin secretion. Collectively, these data support the notion that *PPARGC1A* expression is reduced in animal models of diabetes and human diabetes, and associated with impaired insulin secretion.

The risk of developing type 2 diabetes increases with high-fat/high-energy diets, reduced physical activity and age. However, not all individuals respond to the environment in the same way. This variation in response to environmental factors has partially been ascribed to genetic factors. The common Gly482Ser variant in the *PPARGC1A* gene is a plausible candidate for such a genetic factor [3, 4]. We have previously shown that this polymorphism is associated with an age-related decline in *PPARGC1A* gene expression in human skeletal muscle [5]. The present study shows that the same *PPARGC1A* risk allele, 482Ser, is associated with reduced *PPARGC1A* expression and impaired insulin secretion in human islets. Thus, our results provide the ground for a common genetic predisposition towards type 2 diabetes contributing to both impaired insulin action and secretion.

Reduced *PPARGC1A* expression may not simply reflect an effect of the polymorphism per se but could also occur through epigenetic phenomena such as increased DNA methylation of the *PPARGC1A* promoter. Cytosine residues occurring in CG dinucleotides are targets for DNA methylation, and gene expression is usually reduced when DNA methylation takes place at a promoter. Epigenetic changes are well known to influence gene expression of suppressor genes and oncogenes in cancer cells and contribute to tumour growth [19]. Increased DNA methylation of the promoter of the *PPARGC1A* gene in type 2 diabetic human islets may contribute to beta cell dysfunction by similar mechanisms. To our knowledge, this is the first study to demonstrate that DNA methylation may play a role in regulating gene expression in human diabetic islets.

Our data suggest that PGC-1α can modulate glucose-mediated insulin secretion in human islets, most likely via an effect on ATP production as indicated by normal insulin release in response to arginine and glibenclamide, which stimulate insulin downstream of ATP. In support of such a role, *PPARGC1A* mRNA expression correlated with glucose-stimulated insulin release. Moreover, inhibition of *PPARGC1A* expression by siRNA transfection in human islets was associated with a decline in insulin mRNA and insulin release. Similarly, in human type 2 diabetic islets, reduced *PPARGC1A* mRNA levels were associated with impaired glucose-mediated insulin secretion. The *PPARGC1A* Gly482Ser polymorphism, which previously has been associated with type 2 diabetes, was also associated with reduced *PPARGC1A* expression and impaired insulin secretion in the human islets.

One caveat of this study could be that we did not measure the protein level of PGC-1α in human islets. However, we have previously demonstrated a significant positive correlation between the *PPARGC1A* mRNA and PGC-1α protein levels in human skeletal muscle [5] and we therefore assume that this might also be the case in human islets. The reduction in *PPARGC1A* expression in non-diabetic carriers of a 482Ser allele was strong and factors other than haploinsufficiency may therefore explain this reduction. Moreover, whether the genotype influences *PPARGC1A* expression in diabetic islets would be of interest; however, the present study lacks statistical power to perform this analysis. These relationships should be addressed in future ad hoc studies.

In conclusion, we have shown that *PPARGC1A* mRNA expression is reduced in islets from patients with type 2 diabetes and it is influenced by both genetic (Gly482Ser) and epigenetic (DNA methylation) factors. This reduction in *PPARGC1A* expression correlated with impaired glucose-stimulated insulin secretion, which is known to require ATP. Thereby, the present study provides two novel insights into the molecular mechanisms of islet dysfunction in type 2 diabetes; first that a master transcription regulator of oxidative phosphorylation, PGC-1α, may be involved and second, that epigenetic factors like DNA methylation should be considered when searching for the genetic causes of type 2 diabetes.

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

References

1. Lin J, Handschin C, Spiegelman BM (2005) Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 1:361–370
2. Mootha VK, Lindgren CM, Eriksson KF et al (2003) PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34:267–273

3. Ek J, Andersen G, Urhammer SA et al (2001) Mutation analysis of peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) and relationships of identified amino acid polymorphisms to type II diabetes mellitus. Diabetologia 44:2220–2226
4. Barroso I, Luan J, Sandhu MS et al (2006) Meta-analysis of the Gly482Ser variant in *PPARGC1A* in type 2 diabetes and related phenotypes. Diabetologia 49:501–505
5. Ling C, Poulsen P, Carlsson E et al (2004) Multiple environmental and genetic factors influence skeletal muscle PGC-1alpha and PGC-1beta gene expression in twins. J Clin Invest 114:1518–1526
6. Oberkofler H, Linnemayr V, Weitgasser R et al (2004) Complex haplotypes of the PGC-1alpha gene are associated with carbohydrate metabolism and type 2 diabetes. Diabetes 53:1385–1393
7. Yoon JC, Xu G, Deeney JT et al (2003) Suppression of beta cell energy metabolism and insulin release by PGC-1alpha. Dev Cell 5:73–83
8. Del Guerra S, Lupi R, Marselli L et al (2005) Functional and molecular defects of pancreatic islets in human type 2 diabetes. Diabetes 54:727–735
9. Lupi R, Del Guerra S, Fierabracci V et al (2002) Lipotoxicity in human pancreatic islets and the protective effect of metformin. Diabetes 51(Suppl 1):S134–S137
10. Novelli M, Fabregat ME, Fernandez-Alvarez J, Gomis R, Masiello P (2001) Metabolic and functional studies on isolated islets in a new rat model of type 2 diabetes. Mol Cell Endocrinol 175:57–66
11. Masiello P, Broca C, Gross R et al (1998) Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 47:224–229
12. Galli J, Li LS, Glaser A, Ostenson CG et al (1996) Genetic analysis of non-insulin dependent diabetes mellitus in the GK rat. Nat Genet 12:31–37
13. Fakhrai-Rad H, Nikoshkov A, Kamel A et al (2000) Insulin-degrading enzyme identified as a candidate diabetes susceptibility gene in GK rats. Hum Mol Genet 9:2149–2158
14. Galli J, Fakhrai-Rad H, Kamel A, Marcus C, Norgren S, Luthman H (1999) Pathophysiological and genetic characterization of the major diabetes locus in GK rats. Diabetes 48:2463–2470
15. Lin JM, Ortsater H, Fakhrai-Rad H, Galli J, Luthman H, Bergsten P (2001) Phenotyping of individual pancreatic islets locates genetic defects in stimulus secretion coupling to Niddm1i within the major diabetes locus in GK rats. Diabetes 50:2737–2743
16. Gotoh M, Maki T, Kiyoizumi T, Satomi S, Monaco AP (1985) An improved method for isolation of mouse pancreatic islets. Transplantation 40:437–438
17. Muller YL, Bogardus C, Pedersen O, Baier L (2003) A Gly482Ser missense mutation in the peroxisome proliferator-activated receptor gamma coactivator-1 is associated with altered lipid oxidation and early insulin secretion in Pima Indians. Diabetes 52:895–898
18. Patti ME, Butte AJ, Crunkhorn S et al (2003) Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc Natl Acad Sci U S A 100:8466–8471
19. Herranz M, Esteller M (2006) New therapeutic targets in cancer: the epigenetic connection. Clin Transl Oncol 8:242–249