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ARTICLE

Insulin promoter DNA methylation correlates negatively with insulin gene expression and positively with HbA_{1c} levels in human pancreatic islets

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Abstract
Aims/hypothesis Although recent studies propose that epigenetic factors influence insulin expression, the regulation of the insulin gene in type 2 diabetic islets is still not fully understood. Here, we examined DNA methylation of the insulin gene promoter in pancreatic islets from patients with type 2 diabetes and non-diabetic human donors and related it to insulin expression, HbA_{1c} levels, BMI and age.
Methods DNA methylation was analysed in 25 CpG sites of the insulin promoter and insulin mRNA expression was analysed using quantitative RT-PCR in pancreatic islets from nine donors with type 2 diabetes and 48 non-diabetic donors.
Results Insulin mRNA expression (p=0.002), insulin content (p=0.004) and glucose-stimulated insulin secretion (p=0.04) were reduced in pancreatic islets from patients with type 2 diabetes compared with non-diabetic donors. Moreover, four CpG sites located 234 bp, 180 and 102 bp upstream and 63 bp downstream of the transcription start site (CpG −234, −180, −102 and +63, respectively), showed increased DNA methylation in type 2 diabetic compared with non-diabetic islets (7.8%, p=0.03; 7.1%, p=0.02; 4.4%, p=0.03 and 9.3%, p=0.03, respectively). While insulin mRNA expression correlated negatively ($p<1\times10^{-6}$), the level of HbA_{1c} correlated positively ($p\leq0.01$) with the degree of DNA methylation for CpG −234, −180 and +63. Furthermore, DNA methylation for nine additional CpG sites correlated negatively with insulin mRNA expression ($p\leq0.01$). Also, exposure to hyperglycaemia for 72 h increased insulin promoter DNA methylation in clonal rat beta cells (p=0.005).
Conclusions/interpretations This study demonstrates that DNA methylation of the insulin promoter is increased in patients with type 2 diabetes and correlates negatively with insulin gene expression in human pancreatic islets.

Keywords Alpha cells · Beta cells · DNA methylation · Epigenetic · Gene expression · Human · Hyperglycaemia · Insulin · Pancreatic islets · Type 2 diabetes

Abbreviations

ATF2	Activating transcription factor 2
CREB2	cAMP responsive element binding protein 2
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-α
SNP	Single-nucleotide polymorphism
TFBS	Transcription factor binding site
TSS	Transcription start site

B. T. Yang and T. A. Dayeh contributed equally to this study.

M. D. Nitert and C. Ling contributed equally to this study.

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Introduction

Type 2 diabetes is a multifactorial disease characterised by chronic hyperglycaemia as a result of defects in insulin

secretion from the pancreatic beta cells and insulin action in target tissues including skeletal muscle, adipose tissue and liver. It is well established that genetic and non-genetic factors influence susceptibility to type 2 diabetes. Recent studies suggest that epigenetic factors, including DNA methylation and histone modifications, may influence the pathogenesis of type 2 diabetes [1–4]. Indeed, we have recently shown that DNA methylation of the *PPARGC1A* (encoding peroxisome proliferator-activated receptor γ, coactivator 1 α [PGC-1α]) promoter is increased in pancreatic islets from patients with type 2 diabetes compared with non-diabetic individuals [5]. Moreover, the type 2 diabetic islets showed reduced PGC-1α mRNA levels in parallel with decreased glucose-stimulated insulin secretion. PGC-1α is a transcriptional co-activator that stimulates mitochondrial oxidative metabolism and thereby ATP production. Our study suggests that DNA methylation can regulate gene expression in pancreatic islets from patients with type 2 diabetes and subsequently contribute to impaired insulin secretion [5].

Although previous studies have found the insulin gene to be regulated by epigenetic mechanisms, it is not clear whether epigenetic alterations of the insulin gene are involved in the pathogenesis of impaired insulin secretion in type 2 diabetes [6–8]. A recent study proposed that insulin gene expression is regulated by DNA methylation [9]. Kuroda and co-workers showed that while the degree of DNA methylation is low in the insulin promoter of beta cells, the same promoter is highly methylated in other cell types. By using the insulin promoter in a luciferase expression assay, they further proposed that a high degree of DNA methylation is associated with low gene expression. However, it is not known whether DNA methylation of the insulin promoter differs between pancreatic islets from patients with type 2 diabetes and those from non-diabetic individuals. The aim of the present study was to examine DNA methylation of the insulin promoter in pancreatic islets from 48 non-diabetic and nine type 2 diabetic human donors and to relate the degree of DNA methylation to insulin gene expression, HbA_{1c} levels, BMI and age. We separately analysed insulin promoter DNA methylation in beta and alpha cells isolated from human pancreatic islets as well as in clonal rat beta cells exposed to hyperglycaemia.

Methods

Pancreatic islets Pancreatic islets from 48 non-diabetic and nine type 2 diabetic deceased donors were obtained from the Human Tissue Laboratory at Lund University Diabetes Centre (Table 1). Islets were prepared by collagenase digestion and density gradient purification. After isolation, islets were cultured free floating in CMRL 1066 culture medium (ICN Biomedicals, Costa Mesa, CA, USA) supplemented with 10 mmol/l HEPES, 2 mmol/l L-glutamine, 50 μg/ml gentamicin, 0.25 μg/ml Fungizone (GIBCO BRL, Gaithersburg, MD, USA), 20 μg/ml ciprofloxacin (Bayer Healthcare, Leverkusen, Germany), and 10 mmol/l nicotinamide at 37°C (5% CO_2) prior to RNA and DNA preparation. The islet purity was similar for type 2 diabetic (68±19%) and non-diabetic (58.7±19%, p=0.16) donors. Insulin content in homogenised human islets was assessed by ELISA (Mercodia, Uppsala, Sweden) and values were normalised to the total DNA in each sample as determined by a fluorometric assay (Quant-iT Picogreen, Invitrogen Molecular Probes, Stockholm, Sweden). Glucose-stimulated insulin secretion from the human islets was measured in vitro in static incubations as previously described by Rosengren et al. [10]. The donor before death or her/his relatives had, on admission to the intensive care unit, given their consent to donate organs. The local ethics committees approved the protocols.

Beta cell purification Beta and alpha cells were purified from pancreatic islets of three human donors (aged 54, 55 and 74 years, with BMI 21.5–23.1 kg/m^2), different from the donors described in Table 1, using a method previously described by Parnaud et al. [11]. Dissociation of islet cells was achieved by incubation with constant agitation for 3 min at 37°C in 0.05% (wt/vol.) trypsin-EDTA (Invitrogen) supplemented with 3 mg/ml DNAse I (Roche, Basel, Switzerland) followed by vigorous pipetting. Labelling and FACS sorting of the beta and alpha cell fractions was performed as previously described by Parnaud et al. [11]. Sorted alpha and beta cells were applied to microscope slides and co-immunostained for insulin and glucagon in order to detect the amount of alpha cells in the beta cell fraction, and vice versa. Using this method, a beta cell purity of 89±9% (mean±SD) was achieved [12].

Cell culture Clonal rat insulinoma-derived INS 832/13 beta cells were cultured in RPMI medium with 11.1 mmol/l glucose, which is the basal glucose concentration for these cells, supplemented with 10% fetal calf serum, 1 mmol/l sodium pyruvate, 10 mmol/l HEPES, 50 μmol/l β-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. The cells were plated out in six well plates and allowed to attach overnight. The medium was then replaced with fresh medium containing either 11.1 or 16.7 mmol/l glucose. The cells were further cultured for 72 h, with the medium exchanged after 48 h. DNA was isolated with the All Prep kit (Qiagen, Hilden, Germany) and kept at −20°C until analysis.

Table 1 Characteristics of human pancreatic donors

Characteristic	Type 2 diabetic donors	Non-diabetic donors	p value
n (male/female)	9 (5/4)	48 (26/22)	
Age (years)	57.0±13.1	56.7±10.1	0.9
BMI (kg/m^2)	28.5±4.7	25.4±3.1	0.08
HbA_{1c}	7.3±1.2	5.7±0.8	0.0001

Data are expressed as mean±SD

Gene expression analysis Total RNA was extracted from human islets using All Prep DNA/RNA kit and cDNA was synthesised using QuantiTect Reverse Transcription kit (Qiagen, Hilding, Germany). Insulin mRNA levels were analysed using TaqMan Real-Time PCR with an ABI Prism 7900 HT system and gene-specific probes and primer pairs (Assays-on-demand, Hs02741908_m1; Applied Biosystems, Foster City, CA, USA). The insulin transcript level was normalised to the mRNA level of cyclophilin A (4326316E, Applied Biosystems) and quantified using the ΔΔCt method.

DNA methylation analysis Sequenom's MassARRAY EpiTYPER protocol was applied to measure DNA methylation (Sequenom, San Diego, CA, USA). Five different EpiTYPER assays were designed (EpiDesigner), covering 26 CpG sites of the human insulin promoter (successful data were generated for 25 CpG sites, see below). Also, two assays covering ten CpG sites of the rat insulin promoter were designed (successful data were generated for five CpG sites, see below). The primer sequence and the location of the five human assays and the two rat assays are given in Electronic supplementary material (ESM) Table 1. Genomic DNA was first extracted from the human islets and the clonal rat beta cells using the All Prep DNA/RNA kit (Qiagen) as well as from whole blood cells using the Gentra Puregene Blood Kit (Qiagen). Genomic DNA, 500 ng, was bisulfite treated with the EZ DNA Methylation kit (Zymo Research, Orange, CA, USA). Duplicate PCR reactions were performed with bisulfite-specific PCR primers containing a T7-promotor tag and a 10mer tag on the reverse and forward primer, respectively. In vitro transcription and RNase cleavage reaction were conducted using the MassCleave (hMC) kit according to the manufacturer's recommendations. The cleavage reaction was dispensed onto a 384 element SpectroCHIP bioarray and mass spectra were acquired using a MassARRAY mass spectrometer. The spectra were analysed and the methylation ratios were obtained by the EpiTYPER software v.1.0.1. As a result of the cleavage pattern, four CpG sites were analysed as two separate CpG units (CpG −1,478 together with CpG −1,482 and CpG −1,893 together with CpG −1,897). The results from these CpG units were excluded from further statistical analysis. CpG site −1,565 was excluded from this study because of variation between duplicate samples. CpG site −180 did not generate any methylation data using the EpiTYPER because of low mass of the cleavage product. However, as CpG −180 has previously been suggested to be important for regulation of insulin gene expression via activating transcription factor 2 (ATF2, also known as cAMP responsive element binding protein 2 [CREB2]) binding [9], we used Pyrosequencing (Qiagen) according to manufacturer's recommendations to analyse DNA methylation at CpG −180 (ESM Table 1). Five CpG sites in the rat insulin promoter assay did not generate any methylation data due to either low or high mass of the cleavage product.

Statistical analysis Differences in DNA methylation of the insulin promoter between type 2 diabetes patients and non-diabetic individuals were analysed using multivariate regression analysis including disease status, age, sex and BMI as covariates. Correlations were calculated using the Spearman correlation coefficient. Paired tests were used to analyse differences between beta and alpha cells as well as between clonal rat beta cells cultured in 11.1 or 16.7 mmol/l glucose. Non-parametric two-sample tests were used to analyse differences in insulin mRNA and content as well as glucose-stimulated insulin secretion between individuals with type 2 diabetes and non-diabetic donors. All p values were two-tailed and p values less than 0.05 were considered significant. The presented p values have not been corrected for multiple testing. Statistical calculations were performed using NCSS software (NCSS Statistical Software, Kaysville, UT, USA).

Results

Human pancreatic islets from nine donors with type 2 diabetes and 48 non-diabetic donors were included in this study (Table 1). Insulin mRNA expression, insulin content and glucose-stimulated insulin secretion were decreased in pancreatic islets from individuals with type 2 diabetes compared with those from non-diabetic donors (58% decrease, p=0.002; 57% decrease, p=0.004; and 26% decrease, p=0.04; respectively, Fig. 1). Moreover, the insulin mRNA level correlated positively with insulin content in these islets (rho=0.49, p=0.0004). DNA methylation levels were obtained for 25 CpG sites covering 2,518 bp of the human insulin promoter in the same set of human pancreatic islets (Fig. 2a,b, Table 1 and ESM Table 1). Four CpG sites, located 234, 180, 102 bp upstream (CpG −234, −180, −102) and 63 bp downstream (CpG +63) of the transcription start site, respectively, showed increased DNA methylation in islets from patients

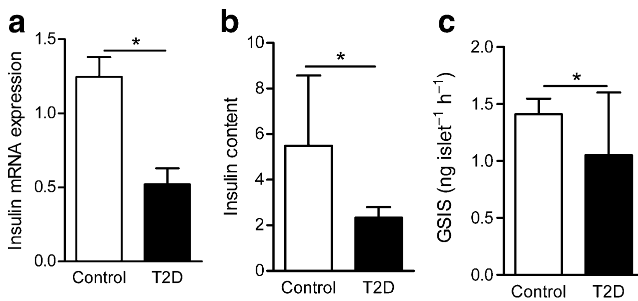

Fig. 1 Insulin mRNA levels, insulin content and glucose-stimulated insulin secretion measured in human pancreatic islets. Pancreatic islets from patients with type 2 diabetes show decreased (**a**) relative insulin mRNA levels, (**b**) insulin content and (**c**) glucose-stimulated insulin secretion, compared with non-diabetic control donors. Results are expressed as mean±SEM. *p<0.05 vs control islets. Control, non-diabetic donors; GSIS, glucose-stimulated insulin secretion; T2D, donors with type 2 diabetes

with type 2 diabetes compared with non-diabetic donors (7.8%, p=0.03; 7.1%, p=0.02; 4.4%, p=0.03 and 9.3%, p = 0.03, respectively, Fig. 3a–d). We next examined whether the degree of methylation of CpG −234, −180, −102 and +63 correlated with insulin gene expression and HbA_{1c} levels in all studied individuals. While insulin mRNA expression correlated negatively, the level of HbA_{1c} correlated positively with DNA methylation at three out of four sites: CpG −234, −180 and +63 (Fig. 4a–d, Fig. 5a–d). By in silico analysis, the 2,518 bp of the insulin promoter sequence was examined for putative transcription factor binding sites (TFBS) using an algorithm from Genomatix (www.genomatix.de, accessed 28 September 2009). Both previously known and putative TFBS that co-localise with the analysed CpG sites are shown in Fig. 2a.

Although there was no significant difference in DNA methylation in islets from type 2 diabetic donors compared with non-diabetic donors for the additional CpG sites analysed, the percentage of DNA methylation correlated negatively with insulin mRNA expression for a number of CpG sites, including CpG −19 (ρ=−0.65, $p<1\times10^{-6}$), CpG −69 (ρ=−0.70, $p<1\times10^{-6}$), CpG −135 (ρ=−0.58, $p=3\times10^{-6}$), CpG −206 (ρ=−0.64, $p<1\times10^{-6}$), CpG −982 (ρ=−0.62, $p<1\times10^{-6}$), CpG −1,014 (ρ=−0.42, p=0.001), CpG −1,695 (ρ=−0.32, p=0.01), CpG −1,926 (ρ=−0.53, $p=3\times10^{-5}$) and CpG −2,254 (ρ=−0.51, $p=6\times10^{-6}$). While both HbA_{1c} levels and BMI correlated negatively with insulin mRNA expression (ρ=−0.39, p=0.008 and ρ=−0.26, p=0.058, respectively), they correlated positively with the level of DNA methylation for a number of insulin CpG sites (HbA_{1c} CpG −19, −69, −135, −206, −982, −1,527, −2,252, ρ=0.29–0.40, p<0.05; and BMI CpG +63, −69, −135, −180, −982, −1,014, −1,527, −1,582, −1,602, −1,695, −1,926, ρ=0.26–0.40, p<0.05). However, there was no significant positive correlation between age and DNA methylation of the insulin promoter.

In order to examine if hyperglycaemia affects insulin promoter DNA methylation specifically in beta cells, we cultured clonal rat insulinoma-derived INS 832/13 beta cells in the presence of either basal (11.1 mmol/l) or high (16.7 mmol/l) levels of glucose for 72 h and measured DNA methylation for five CpG sites of the rat insulin promoter. Hyperglycaemia increased DNA methylation significantly for two specific CpG sites located 1,057 bp upstream of the transcription start site (TSS) (CpG −1,057, 12.7% increase, p=0.005) and 58 bp downstream of the TSS (CpG +58, 5.0% increase, p=0.03) in the clonal rat beta cells (Fig. 5e). Furthermore, the average DNA methylation of the studied insulin promoter region increased in rat clonal beta cells exposed to hyperglycaemia (8.3% increase, p=0.047, Fig. 5e).

We proceeded to compare the overall DNA methylation patterns in human pancreatic islets and blood cells. In human pancreatic islets, the methylation level of the analysed CpG sites varied according to the distance from the TSS. In general, CpG sites located closer to the TSS showed lower levels of DNA methylation compared with CpG sites located further upstream (5′) (Fig. 2b). Indeed, the average methylation level for CpG sites +63 to −234 was significantly lower than the average methylation level for CpG sites −982 to −2,266 (34.3±11.5% vs 73.9±12.4%, p<0.0001). In contrast, in blood cells DNA methylation was high throughout the analysed insulin promoter and for the majority of CpG sites the methylation level was higher in blood than in human islets (Fig. 2b). Moreover, when comparing insulin promoter DNA methylation in beta and alpha cells isolated from human pancreatic islets, five out of eight analysed CpG sites showed lower methylation in beta than in alpha cells (p<0.05) (Fig. 2c).

Finally, as it has been proposed that CpG sites close to each other show a similar degree of methylation within a tissue and individual, we also tested if the percentage of insulin promoter methylation of the analysed CpG sites correlated in the pancreatic islets. Indeed, the degree of methylation correlated for the majority of analysed CpG sites, i.e. all the first eight CpG sites analysed correlated positively, including CpG +63, −19, −69, −102, −135, −180, −206, −234 (ρ=0.66–0.91, $p<1\times10^{-6}$).

Discussion

This study proposes that DNA methylation affects insulin mRNA expression levels in human pancreatic islets. Four CpG sites, located 234, 180, 102 upstream (5′) and 63 bp downstream (3′) of the transcription start site of the insulin gene (CpG −234, −180, −102 and +63), had a higher degree of DNA methylation in islets from type 2 diabetes

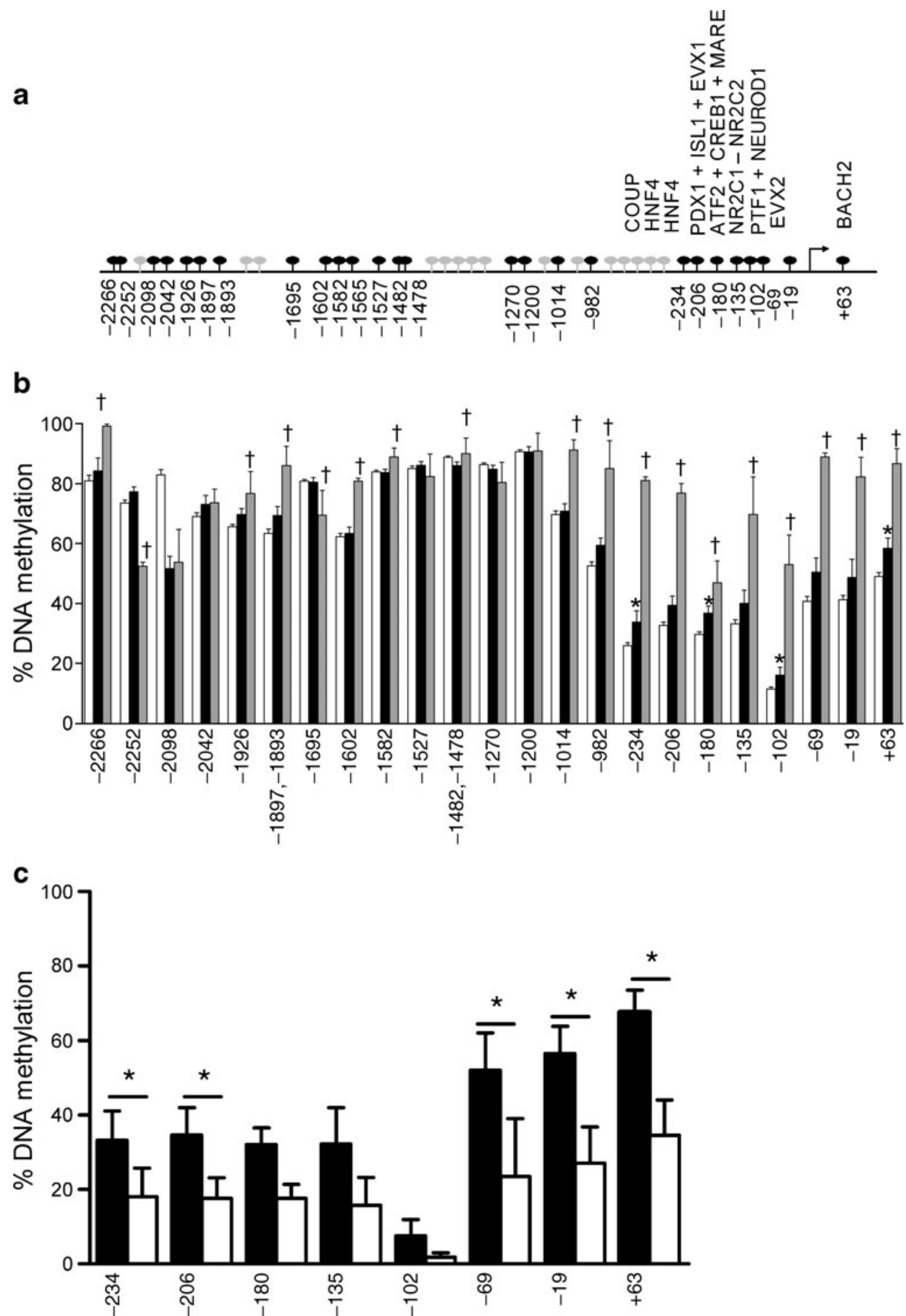

Fig. 2 DNA methylation of the insulin promoter. (**a**) A schematic representation of 2,518 bp of the insulin gene region analysed for DNA methylation. Positions of CpG sites in relation to the TSS are indicated: black circles represent analysed CpG sites, grey circles are not analysed. Putative and previously known transcription factor binding sites that co-localise with a CpG site are indicated. DNA methylation levels in percentage are analysed in (**b**) human pancreatic islets from 48 controls (white bars) and nine type 2 diabetic patients (black bars) as well as in five blood samples (grey bars; one man and four women, 29–57 years) and (**c**) beta cells (white bars) and alpha cells (black bars) isolated from pancreatic islets of three human donors. Results are expressed as mean±SEM, and have not been corrected for multiple testing. $^{\dagger}p<0.05$ vs control islets, $*p<0.05$ vs control islets. BACH2, basic leucine zipper transcription factor 2; COUP, chicken ovalbumin upstream promoter; HNF4, hepatocyte nuclear factor 4; PDX1, pancreatic and duodenal homeobox 1; ISL1, islet 1; EVX1/2, even-skipped homeobox 1/2; MARE, Maf recognition element; NR2C1/2, nuclear receptor subfamily 2, group C, member 1/2; PTF1, pancreas specific transcription factor, NEUROD1, neurogenic differentiation 1

patients than in islets from non-diabetic controls. Higher DNA methylation of the insulin promoter inversely correlated with lower insulin mRNA levels. Moreover, both insulin mRNA expression and insulin content as well as glucose-stimulated insulin secretion were decreased in islets from type 2 diabetic donors.

Previous studies together with the present study show that differences in DNA methylation between type 2 diabetic patients and non-diabetic controls are less than 10% [4, 5]. Type 2 diabetes is a polygenic disease and it is possible that the effects of DNA methylation on type 2 diabetes are similar to those seen in genetic studies, where multiple loci with small effect sizes together contribute to an increased risk for disease [13–16]. One limit with our study is the small number of diabetic islets, and replication studies in other cohorts are needed to confirm our results. Future genome-wide methylation studies with larger number of patients are further required to dissect the role of

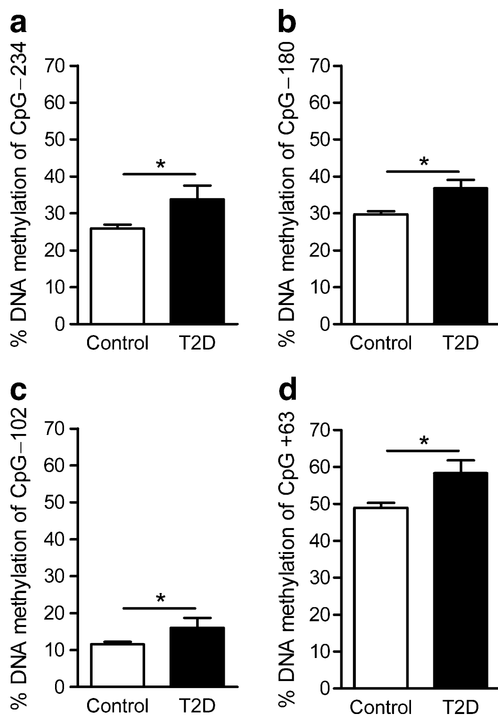

Fig. 3 Impact of type 2 diabetes on DNA methylation of the insulin promoter. Pancreatic islets from nine patients with type 2 diabetes show elevated insulin promoter DNA methylation in (**a**) CpG site −234, (**b**) CpG site −180, (**c**) CpG site −102 and (**d**) CpG site +63, compared with 48 non-diabetic donors. Results are expressed as mean±SEM and have not been corrected for multiple testing. *$p<0.05$. Control, non-diabetic donors; T2D, donors with type 2 diabetes

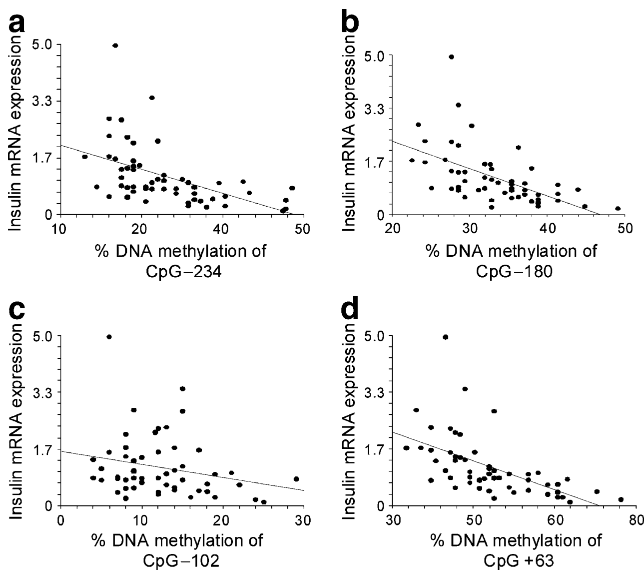

Fig. 4 Correlations between the level of insulin mRNA expression and insulin promoter DNA methylation in human pancreatic islets. The relative insulin mRNA expression correlates negatively with insulin promoter DNA methylation at (**a**) CpG site −234 (ρ=−0.64, $p<1\times10^{-6}$), (**b**) CpG site −180 (ρ=−0.66, $p<1\times10^{-6}$) and (**d**) CpG site +63 (ρ=−0.72, $p<1\times10^{-6}$), while borderline significance was established for (**c**) CpG site −102 (ρ=−0.25, p=0.065)

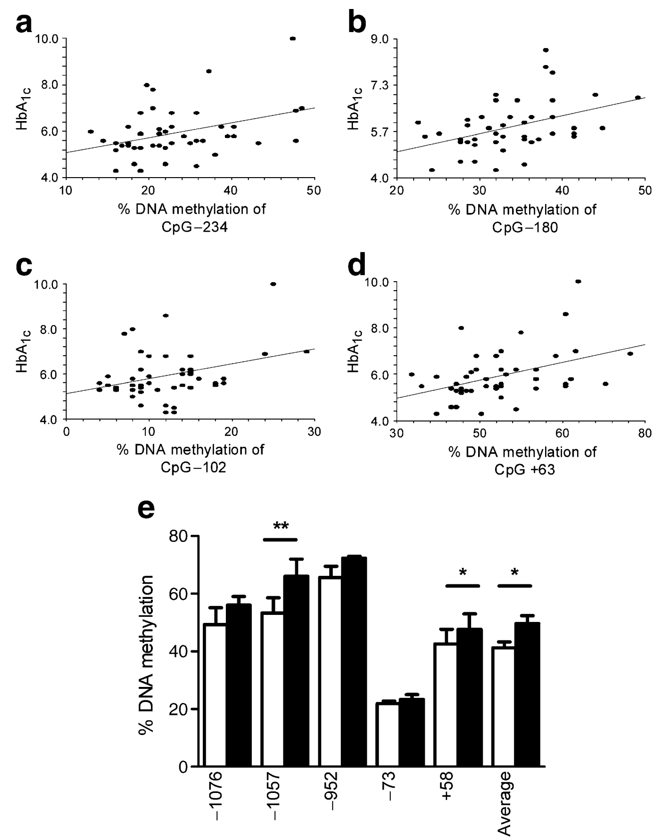

Fig. 5 Impact of hyperglycaemia on insulin promoter DNA methylation at (**a**) CpG site −234, (**b**) CpG site −180, (**c**) CpG site −102 and (**d**) CpG site +63. The HbA_{1c} level correlates positively with insulin promoter DNA methylation at (**a**) CpG site −234 (ρ=0.36, p=0.01), (**b**) CpG site −180 (ρ=0.42, p=0.004) and (**d**) CpG site +63 (ρ=0.44, p=0.002), while borderline significance was established for (**c**) CpG site −102 (ρ=0.28, p=0.056) in human pancreatic islets. (**e**) Clonal rat beta cells cultured in 16.7 mmol/l glucose showed increased insulin promoter DNA methylation of CpG sites −1,057 and +58 and a significant increase was also seen for the average DNA methylation measured across the studied region compared with beta cells cultured in 11.1 mmol/l glucose. Results are expressed as mean±SEM. *$p<0.05$, **$p<0.01$. Black bars, beta cells cultured in 16.7 mmol/l glucose; white bars, beta cells cultured in 11.1 mmol/l glucose

DNA methylation in islets from individuals with type 2 diabetes.

Interestingly, elevated HbA_{1c} levels were associated with increased DNA methylation of the human insulin promoter. Recent studies have demonstrated that hyperglycaemia may induce epigenetic changes and thereby increase expression of pro-inflammatory genes in vascular cells, which subsequently may increase the risk for diabetic complications [17–20]. It is possible that hyperglycaemia also induces epigenetic changes, e.g. increases DNA methylation, in the insulin promoter of human beta cells, resulting in reduced insulin expression. Indeed, our data support such a hypothesis; we found that hyperglycaemia for 72 h increased insulin promoter DNA methylation in clonal rat beta cells. Although we cannot rule out that the changes we

find in insulin promoter DNA methylation in type 2 diabetic islets are due to altered cell composition in islets from type 2 diabetic donors, our data from the clonal rat beta cells demonstrate that hyperglycaemia specifically increases insulin promoter DNA methylation in beta cells.

Age and obesity are important risk factors for type 2 diabetes. Although our previous studies have shown that ageing is associated with increased DNA methylation and reduced gene expression of type 2 diabetes candidate genes in human skeletal muscle, we did not find any correlation between age and DNA methylation of the insulin promoter in pancreatic islets [1, 3]. Nevertheless, a lack of correlation between age and DNA methylation could reflect the small number of young donors included in this study, as only three donors were younger than 40 years. However, increased BMI was associated with reduced insulin expression and increased DNA methylation, suggesting that obesity may increase methylation of the insulin promoter.

Two studies have recently analysed histone modifications and DNA methylation of the insulin gene in human pancreatic islets and in isolated pancreatic beta cells [8, 9]. They investigated islets from a small number of donors, and no islets from type 2 diabetic patients were included. In agreement with our data, they found lower levels of DNA methylation in pancreatic islets compared with other cell types. Interestingly, when we analysed DNA methylation in isolated human beta cells we found, on average, 19% DNA methylation, demonstrating that the insulin promoter of isolated beta cells is not completely unmethylated. Moreover, when Kuroda and co-workers performed chromatin immunoprecipitation (ChIP) experiments in cells transfected with either methylated or unmethylated insulin promoter sequences, they found that methylation inhibited binding of ATF2 to the insulin promoter and it increased binding of a methyl-binding protein, methyl CpG binding protein 2 (MeCP2), which is associated with reduced transcription [9]. Together, these studies suggest that DNA methylation can regulate insulin expression in beta cells. However, factors additional to our observed changes in DNA methylation in type 2 diabetic islets could contribute to the changes found in insulin gene expression. It has been proposed that DNA methylation of CpG sites close to each other show a similar degree of methylation. Although our study supports this theory, the mechanisms behind this phenomenon are unknown.

In conclusion, four CpG sites within the insulin promoter show increased DNA methylation in pancreatic islets from patients with type 2 diabetes compared with non-diabetic donors. Moreover, hyperglycaemia may increase insulin promoter DNA methylation in beta cells. Our study further suggests that DNA methylation can influence insulin gene expression in human pancreatic islets.

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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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References

1. Ling C, Poulsen P, Simonsson S et al (2007) Genetic and epigenetic factors are associated with expression of respiratory chain component NDUFB6 in human skeletal muscle. J Clin Invest 117:3427–3435
2. Ling C, Groop L (2009) Epigenetics: a molecular link between environmental factors and type 2 diabetes. Diabetes 58:2718–2725
3. Ronn T, Poulsen P, Hansson O et al (2008) Age influences DNA methylation and gene expression of COX7A1 in human skeletal muscle. Diabetologia 51:1159–1168
4. Barres R, Osler ME, Yan J et al (2009) Non-CpG methylation of the PGC-1alpha promoter through DNMT3B controls mitochondrial density. Cell Metab 10:189–198
5. Ling C, Del Guerra S, Lupi R et al (2008) Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. Diabetologia 51:615–622
6. Chakrabarti SK, Francis J, Ziesmann SM, Garmey JC, Mirmira RG (2003) Covalent histone modifications underlie the developmental regulation of insulin gene transcription in pancreatic beta cells. J Biol Chem 278:23617–23623
7. Mutskov V, Raaka BM, Felsenfeld G, Gershengorn MC (2007) The human insulin gene displays transcriptionally active epigenetic marks in islet-derived mesenchymal precursor cells in the absence of insulin expression. Stem Cells 25: 3223–3233
8. Mutskov V, Felsenfeld G (2009) The human insulin gene is part of a large open chromatin domain specific for human islets. Proc Natl Acad Sci USA 106:17419–17424
9. Kuroda A, Rauch TA, Todorov I et al (2009) Insulin gene expression is regulated by DNA methylation. PLoS ONE 4:e6953
10. Rosengren AH, Jokubka R, Tojjar D et al (2010) Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. Science 327:217–220
11. Parnaud G, Bosco D, Berney T et al (2008) Proliferation of sorted human and rat beta cells. Diabetologia 51:91–100
12. Kirkpatrick CL, Marchetti P, Purrello F et al. Type 2 diabetes susceptibility gene expression in normal or diabetic sorted human alpha and beta cells: correlations with age or BMI of islet donors. PLoS ONE 5:e11053

13. Saxena R, Voight BF, Lyssenko V et al (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316:1331–1336
14. Sladek R, Rocheleau G, Rung J et al (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445:881–885
15. Zeggini E, Weedon MN, Lindgren CM et al (2007) Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science 316:1336–1341
16. Scott LJ, Mohlke KL, Bonnycastle LL et al (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316:1341–1345
17. El-Osta A, Brasacchio D, Yao D et al (2008) Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med 205:2409–2417
18. Brasacchio D, Okabe J, Tikellis C et al (2009) Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that coexist on the lysine tail. Diabetes 58:1229–1236
19. Miao F, Gonzalo IG, Lanting L, Natarajan R (2004) In vivo chromatin remodeling events leading to inflammatory gene transcription under diabetic conditions. J Biol Chem 279:18091–18097
20. Miao F, Smith DD, Zhang L, Min A, Feng W, Natarajan R (2008) Lymphocytes from patients with type 1 diabetes display a distinct profile of chromatin histone H3 lysine 9 dimethylation: an epigenetic study in diabetes. Diabetes 57:3189–3198