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ARTICLE

Increased expression of miR-187 in human islets from individuals with type 2 diabetes is associated with reduced glucose-stimulated insulin secretion

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Abstract
Aims/hypothesis Type 2 diabetes is characterised by progressive beta cell dysfunction, with changes in gene expression playing a crucial role in its development. MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression and therefore alterations in miRNA levels may be involved in the deterioration of beta cell function.
Methods Global TaqMan arrays and individual TaqMan assays were used to measure islet miRNA expression in discovery ($n=20$) and replication ($n=20$) cohorts from individuals with and without type 2 diabetes. The role of specific dysregulated miRNAs in regulating insulin secretion, content and apoptosis was subsequently investigated in primary rat islets and INS-1 cells. Identification of miRNA targets was assessed using luciferase assays and by measuring mRNA levels.
Results In the discovery and replication cohorts miR-187 expression was found to be significantly increased in islets from individuals with type 2 diabetes compared with matched controls. An inverse correlation between miR-187 levels and glucose-stimulated insulin secretion (GSIS) was observed in islets from normoglycaemic donors. This correlation paralleled findings in primary rat islets and INS-1 cells where overexpression of miR-187 markedly decreased GSIS without affecting insulin content or apoptotic index. Finally, the gene encoding homeodomain-interacting protein kinase-3 (*HIPK3*), a known regulator of insulin secretion, was identified as a direct target of miR-187 and displayed reduced expression in islets from individuals with type 2 diabetes.
Conclusions/interpretation Our findings suggest a role for miR-187 in the blunting of insulin secretion, potentially involving regulation of *HIPK3*, which occurs during the pathogenesis of type 2 diabetes.

Keywords Glucose-stimulated insulin secretion · HIPK3 · Islets · MicroRNA · Type 2 diabetes

Abbreviations

GSIS	Glucose-stimulated insulin secretion
HIPK3	Homeodomain-interacting protein kinase-3
miRNA	MicroRNA
sn	Small nuclear

Introduction

In type 2 diabetes the inability of beta cells to compensate for reduced insulin sensitivity is associated with specific changes in gene expression, which may conceivably play a causal role in beta cell dysfunction. MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression by binding to target mRNAs, resulting in mRNA decay and/or translational repression. There is growing evidence that changes in the expression of miRNAs can affect beta cell function (reviewed by Guay et al [1]). Recent evidence has suggested that changes in miRNA expression may either precede diabetes onset, and be associated with positive effects

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on beta cell function, or occur on manifestation of the disease and impact negatively on beta cell function [2, 3]. Indeed, a comparison of the islet miRNome of the Goto–Kakizaki (GK) rat model of type 2 diabetes, with Wistar controls, has revealed a set of upregulated miRNAs enriched in targets within pathways known to be involved in disease causation [4]. In the present study we hypothesised that a similar strategy, measuring the expression of miRNAs in human islets from individuals with and without type 2 diabetes, might identify specific miRNAs with a causal role in human beta cell dysfunction.

Methods

Human islet tissue For global miRNA profiling snap-frozen human islets were received from three centres (National Disease Research Interchange, Philadelphia, PA, USA; AMS Biotechnology, Abingdon, UK; and ProCell Biotech, Newport Beach, CA, USA). All islets were taken with approval from appointed ethics committees. For replication all islets came from ProCell Biotech. Islet purity and viability were determined by dithizone and fluorescein diacetate/propidium iodide staining, respectively [5, 6]. Islets from ProCell Biotech were provided with information regarding glucose-stimulated insulin secretion (GSIS), determined as follows. After isolation and overnight culture islets were incubated sequentially in media containing low (2.8 mmol/l) and high (28 mmol/l) glucose for 1 h. The amount of insulin present in each supernatant fraction was measured by electrochemiluminescence immunoassay on a Cobas e601 analyser (Roche, Indianapolis, IN, USA). GSIS was calculated as insulin secretion at 28 mmol l^{-1}/2.8 mmol l^{-1} glucose.

Isolation and culture of primary rat islets and INS-1 cells The rat insulinoma cell line INS-1 was cultured in RPMI 1640 GlutaMAX (Life Technologies, Paisley, UK) media supplemented with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin and 100 μmol/l β-mercaptoethanol. Primary rat islets were isolated by collagenase digestion [7], hand-picked and cultured, prior to transfection, for at least 24 h in RPMI 1640 medium containing 10 mmol/l glucose, 10% FCS, 100 U/ml penicillin and 100 μg/ml streptomycin.

RNA extraction and real-time quantitative PCR Total RNA, including miRNA, was extracted using the miRVana miRNA isolation kit (Life Technologies). MiRNA reverse transcription reactions were carried out using the miRNA reverse transcription kit (Life Technologies). For global profiling human TaqMan arrays (version 2.0, cards A + B containing assays to 667 miRNAs; Life Technologies) were used with a pre-amplification step included, following the manufacturers' instructions. Values for C_t were generated using automatic settings, ΔC_t was calculated using a global mean normalisation strategy [8], and $\Delta\Delta C_t$ for each gene was determined using a mean ΔC_t value in control samples. Profiling of miR-187, miR-345, miR-15b and U6 small nuclear (sn)RNA was conducted using inventoried TaqMan miRNA assays (Life Technologies) with expression determined from three separate reverse transcription reactions. For the measurement of rat *Hipk3* and human *HIPK3* expression, RNA was reverse transcribed using SuperScript III First-Strand Synthesis System (Life Technologies) with oligo-dT priming. For rat *Hipk3* expression RNA was, prior to reverse transcription, DNased using the Turbo DNA-*free* kit (Life Technologies). TaqMan Gene Expression Assays (Life Technologies) were used to measure expression of *Hipk3* (Rn00582409_m1) and *HIPK3* (Hs00178628_m1). Expression was normalised using GeNorm [9], with the following housekeeping genes measured where appropriate; *Ubc* (Rn01789812_g1), *Tbp* (Rn01455648_m1), *Hprt1* (Rn01527840_m1), *B2M* (Hs00984230), *GUSB* (Hs00939627) and *RPLP0* (Hs99999902_m1). Relative expression was calculated using the comparative C_t method. All reactions were run on an ABI7900HT platform (Life Technologies).

INS-1 transfection and measurement of insulin secretion and beta cell apoptosis Transient transfection of INS-1 cells, at a density of ~2×10^6 cells, was conducted with miRVana miRNA mimics (Life Technologies) and a Nucleofector Device (Lonza, Basel, Switzerland). Negative Control miRVana miRNA mimic number 1 (Life Technologies), which has been designed not to target any known human, mouse or rat gene, was used as a negative control. Transfected cells were plated in 24-well poly-D-lysine-coated plates at a density of ~3×10^5 cells/well. After 48 h, media were removed and cells washed once and then incubated for 2 h in modified Krebs–Ringer medium (125 mmol/l NaCl, 4.74 mmol/l KCl, 1 mmol/l $CaCl_2$, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 5 mmol/l $NaHCO_3$ and 25 mmol/l Hepes, pH 7.4) containing 0.1% BSA and 2.8 mmol/l glucose. Cells were then subjected to either high (28 mmol/l) or low (2.8 mmol/l) glucose treatment for 1 h before the supernatant fraction was removed for insulin determination. Levels of insulin were measured by radioimmunoassay (Linco Research, St Charles, MO, USA) and normalised to protein content as determined by BCA assay (Pierce, Rockford, IL, USA). For the analysis of INS-1 apoptosis 48 h post-transfection the ApopTag Fluorescein Direct In Situ Apoptosis Detection Kit (Millipore, Billerica, MA, USA) was used according to the manufacturer's instructions. Samples were co-stained with DAPI, mounted in VectorShield (Vector Laboratories, Peterborough, UK) and viewed using a Zeiss AxioObserver Z1 microscope (Carl

Zeiss, Oberkochen, Germany) equipped with a ×40 1.3 numerical aperture (NA) oil objective and controlled by AxioVision software (Carl Zeiss) As a positive control untransfected cells were heat shocked at 56°C for 3 min. The cells were allowed to recover at 37°C for 1 h before the assay was performed. Heat shock induced ~40% of cells to become apoptotic (data not shown).

Primary rat islet transfection and measures of insulin secretion and beta cell apoptosis Transfections were carried out using TransIT-TKO (Mirus Bio Corporation, Madison, WI, USA) in the presence of 1 nmol/l miRVana miRNA mimic (Life Technologies) for 48 h prior to assays. Insulin secretion was determined as previously described [10] with insulin content assayed following acidified ethanol extraction. We note that while transfection of the intact islet is likely to affect only the outermost layers of cells [11] it is from these that the majority of stimulated insulin secretion is likely to be observed in vitro given the loss of islet vasculature which occurs rapidly during culture [12]. For the analysis of apoptosis, islets were fixed in 4% paraformaldehyde and stained using the DeadEnd fluorometric TUNEL system (Promega, Madison, WI, USA), as per the manufacturer's protocol for non-adherent cells. Following nicked end labelling using the kit, islets were washed in PBS and then incubated overnight at 4°C with guinea pig anti-insulin antibody (1:200; Dako, Glostrup, Denmark) in PBS containing 0.1% Triton X-100 and BSA. Islets were then washed and incubated in goat anti-guinea pig Alexa Fluor 568 (1:1,000; Life Technologies) in PBS for 1 h at room temperature. Subsequently, islets were washed twice in PBS and spotted on superfrost slides. The slides were left to set overnight in Vectashield HardSet Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA, USA) at room temperature in the dark. Islets were imaged using a Zeiss Axiovert-200 confocal microscope with an Improvision/Nokigawa spinning disc, and running Volocity 5.0 (Improvision, Coventry, UK) software. Image analysis was performed using ImageJ v.1.43m (http://rsbweb.nih.gov/ij/download.html).

Luciferase assay The pMirTarget plasmids containing the 3′ UTR of human *HIPK3* (NM_001048200) downstream of firefly luciferase, and a mutant version differing only by a C-to-G substitution (underlined) within the predicted miR-187 binding site (UUCUAACUAGUGCAAGAC ACGU), were purchased (Origene, Rockville, MD, USA). Luciferase activities were measured using the Dual-Glo Luciferase Assay System (Promega). To account for differences in transfection efficiency firefly luciferase activity was normalised to *Renilla* expression which originated from co-transfected pRL-SV40 (Promega).

Statistical analysis Except where stated otherwise, statistical differences were assessed using two-tailed one-sample or two-sample *t* tests, with correction for multiple testing as indicated. A p value <0.05 was considered significant. All data are presented as mean ± SEM.

Results

miR-187 expression is increased in islets from donors with type 2 diabetes The global miRNA profile of islets from 20 donors (11 with type 2 diabetes, nine controls) was determined using TaqMan arrays. Of the 667 miRNA assays on these arrays 255 amplified in all samples (electronic supplementary material [ESM] Table 1) and the use of pre-amplification meant we decided to limit our analysis to these miRNAs (we found many 'on/off' changes in expression, most likely due to inconsistent amplification of very weakly expressed miRNAs). Two miRNAs, miR-187 and miR-345, displayed higher and statistically significant (after Benjamini–Hochberg false discovery rate correction) differential expression between islets from donors with and without type 2 diabetes (Table 1).

We next sought to replicate our results in an independent cohort using individual miRNA-specific TaqMan assays. We measured the expression of miR-345 and miR-187 in another 20 islet samples (ten from individuals with type 2 diabetes, ten without diabetes) and again found significantly higher miR-187 expression in islets from donors with type 2 diabetes vs healthy donors. The increase in miR-345 islet expression observed in individuals with type 2 diabetes in the initial cohort did not replicate (Table 1). No significant differences in age, sex, BMI, ethnicity, islet purity or islet viability between groups were identified in either islet cohort. Also consistent with previous findings [13] and with a primary role for reduced GSIS in disease pathogenesis, islets in the replication cohort from individuals with type 2 diabetes displayed reduced glucose-stimulated insulin release when compared with islets from individuals without diabetes (Table 2).

miR-187 expression is inversely correlated with GSIS Given the observed increase in miR-187 expression in both islet cohorts we investigated whether this miRNA might play a role in islet function or survival. In islets from 35 normoglycaemic donors we found a significant inverse correlation between miR-187 expression and GSIS (Fig. 1a). No significant correlation was found between levels of miR-15b and GSIS (data not shown), despite levels being similarly normalised to U6 snRNA.

Overexpression of miR-187 in islets and beta cells reduces GSIS To determine whether increased miR-187 expression

Table 1 Results of miRNA expression profiling in human islets from individuals with and without type 2 diabetes

miRNA	Global TaqMan array profiling			Individual TaqMan assay profiling	
	Relative expression[a]	Unadjusted *p* value	Adjusted *p* value[b]	Relative expression[a]	Unadjusted *p* value
miR-187	7.55	7×10^{-5}	0.009	5.38	0.021
miR-345	1.92	2×10^{-5}	0.006	0.64	0.112
miR-129-3p	3.32	0.002	0.115	ND	ND

[a] Calculated using the formula: mean expression in those with type 2 diabetes/mean expression in controls

[b] Adjusted using Benjamini–Hochberg false discovery rate

Statistical significance determined using two-sample *t* tests and Fisher's exact test

ND, not determined

might contribute directly to reduced GSIS, miR-187 mimics or control sequences were transiently transfected into primary rat islets. Compared with mimic-control-transfected islets the introduction of miR-187 mimics sharply reduced insulin secretion stimulated by high (20 mmol/l) glucose and to a far lesser, albeit significant degree, on KCl stimulation (Fig. 1b). There were no significant differences in insulin content between miR-187 and mimic-control-transfected islets (Fig. 1c).

We next tested whether the above actions of miR-187 were likely to be through a cell-autonomous effect of miR-187 on beta cells within the islet. Correspondingly, measured in the rat pancreatic beta cell line, INS-1, near-physiological levels of miR-187 overexpression (ten- to 20-fold increase in cells transfected with miR-187 compared with mimic control; ESM Fig. 1a) also resulted in a significant reduction in insulin secretion under high (28 mmol/l) but not low (2.8 mmol/l) glucose conditions, compared with cells transfected with mimic control (ESM Fig. 1b).

Transfection of miR-187 mimic into primary rat islets and INS-1 cells resulted in no significant difference in rates of apoptosis, as assessed by TUNEL assay, when compared with mimic-control-transfected cells (ESM Fig. 2), and no evident effects on cell viability as judged through overall cellular morphology (data not shown).

HIPK3 is a direct target of miR-187 In order to identify putative miR-187 targets we used miRWalk[14], a database that compiles results from multiple commonly used prediction programs (TargetScan, miRanda, miRDB and RNA22). One of the putative targets identified was the gene encoding homeodomain-interacting protein kinase-3 (*HIPK3*), a known regulator of insulin secretion [15]. Strikingly, the blunted insulin secretory responses at high glucose concentrations in *Hipk3*$^{-/-}$ mice, or on small interfering (si)RNA-mediated knockdown of HIPK3 in isolated mouse islets [15], are similar to the phenotype we observe on miR-187 overexpression.

Table 2 Clinical characteristics of donors in discovery and replication cohorts

Characteristic	Global TaqMan array profiling			Individual TaqMan assay profiling		
	T2D islets	Control islets	*p* value	T2D islets	Control islets	*p* value
n	9	11	–	10	10	–
Sex (male/female)	7/2	5/6	0.20	3/7	5/5	0.65
Ethnicity (white/African–American/Asian)	5/2/2	9/2/0	0.19	4/6/0	9/1/0	0.06
Age (years)	53 (8)	47 (9)	0.09	55 (9)	51 (6)	0.24
BMI	36 (14)	30 (6)	0.27	32 (4)	29 (5)	0.08
Islet purity (%)	81 (8)	82 (10)	0.94	89 (5)	90 (5)	0.51
Islet viability (%)	90 (6)	88 (8)	0.58	91 (2)	92 (2)	0.30
Cold ischaemic time (h)	14 (7)	16 (5)	0.54	16 (6)	15 (6)	0.61
GSIS[a]	ND	ND	–	2.6 (1.3)	4.2 (1.4)	0.02

[a] Calculated using formula: insulin secretion at 28 mmol/l glucose/insulin secretion at 2.8 mmol/l glucose

Where appropriate, data presented as mean (SD)

Statistical significance determined using two-sample *t* tests and Fisher's exact test

ND, not determined; T2D, type 2 diabetes

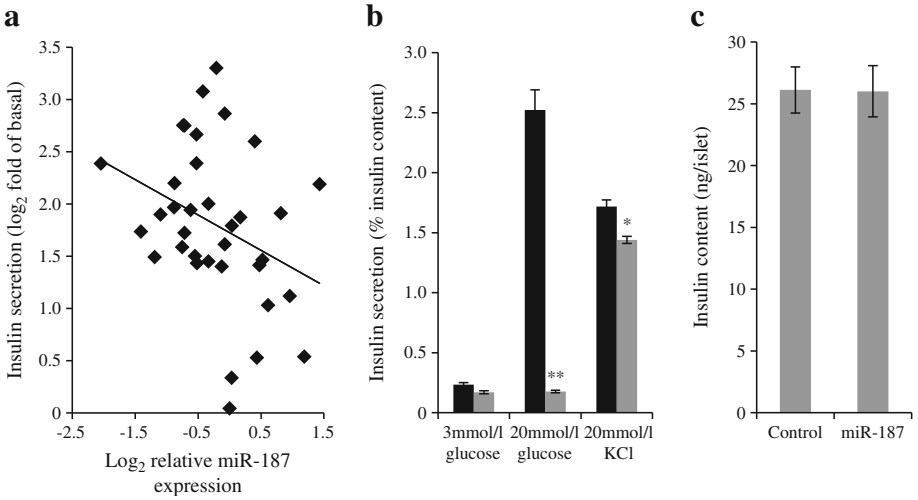

Fig. 1 Increased miR-187 expression is associated with reduced GSIS. (**a**) In islets from 35 non-diabetic donors higher levels of miR-187 expression correlated with reduced GSIS (calculated as amount of insulin secreted at 28 mmol l^{-1}/amount of insulin secreted at 2.8 mmol l^{-1}). miRNA expression was determined from three separate reverse transcriptions using real-time PCR. Statistical significance was assessed by the Pearson correlation coefficient test; $r=-0.34$, $p=0.049$. (**b**) Compared with mimic-control-transfected cells, the introduction of miR-187 mimic into primary rat islets reduced insulin secretion under high glucose (20 mmol/l) conditions. $^{**}p<0.01$ and $^{*}p<0.05$ vs cells transfected with mimic control, $n=3$ independent experiments. Black, control; grey, miR-187. (**c**) No significant difference in insulin content in primary rat islets transfected with control and miR-187 mimics, $n=3$ independent experiments. All data expressed as mean ± SEM

To explore the possibility that HIPK3 may be regulated by miR-187 we first measured *Hipk3* mRNA levels in INS-1 cells transfected with miR-187 mimic and mimic control. The putative miR-187 binding site is conserved from human to rat so a decrease in *Hipk3* expression was expected. Indeed, as determined by real-time qPCR, *Hipk3* mRNA levels were significantly reduced in INS-1 cells transfected with miR-187 mimic compared with mimic-control-transfected cells (Fig. 2a). Furthermore, overexpression of miR-187 in INS-1 cells significantly inhibited the expression of a construct in which the 3′ UTR sequence of human *HIPK3* was fused downstream of luciferase cDNA. This inhibition was not seen when the predicted miR-187 target sequence was mutated, indicating a direct interaction between miR-187 and the 3′ UTR of human *HIPK3* (Fig. 2b). Subsequent measurement of *HIPK3* transcripts in the islets comprising the discovery and replication cohorts for the miRNA profiling also revealed a reduction of *HIPK3* mRNA expression in islets from individuals with type 2 diabetes vs those without (Fig. 2c).

Discussion

Despite concerns regarding the effect of hyperglycaemia on transcript levels, differential mRNA expression in human islets from donors with type 2 diabetes vs those without diabetes can successfully identify genes with a causal role in beta cell dysfunction [16]. Using a similar global profiling approach we have identified a specific miRNA, miR-187, with reproducibly higher expression in islets from donors with type 2 diabetes. We have consequently elucidated a role for miR-187 in regulating insulin secretion, possibly through direct targeting of *HIPK3*.

Many previous studies describing differential islet gene expression from individuals with and without type 2 diabetes have been plagued by a failure to replicate. One of the

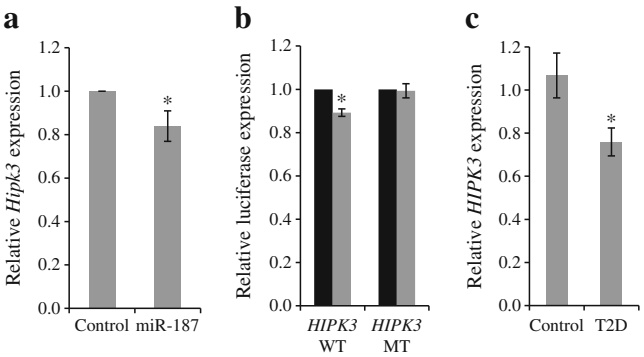

Fig. 2 *HIPK3* is a direct target of miR-187. (**a**) In INS-1 cells transfected with miR-187 mimic endogenous *Hipk3* mRNA expression is reduced, $^{*}p<0.05$ vs cells transfected with negative control miRNA mimic. Statistical significance assessed by one-tailed one-sample *t* test, $n=8$ independent experiments. (**b**) In INS-1 cells overexpression of miR-187 inhibited luciferase expression from a construct containing the 3′ UTR sequence of human *HIPK3* (WT), but not expression from a construct containing the 3′ UTR sequence of human *HIPK3* where the putative miR-187 binding site has been mutated (MT). Statistical significance assessed by one-sample *t* test; $n=4$ independent experiments. Black, control; grey, miR-187. (**c**) *HIPK3* mRNA expression is reduced in islets from individuals with type 2 diabetes (T2D) ($n=17$) compared with islets from matched controls ($n=18$). Statistical significance assessed by two-sample *t* test. All data presented as mean ± SEM

strengths of our study was the use of a second islet cohort, the prudence of such an approach highlighted by differential miR-345 expression not replicating. A failure to match groups for age, BMI, sex, ethnicity, islet purity and viability can also lead to spurious results. In the present study there were no significant differences in such confounding factors, all of which can influence mRNA expression [17, 18], and are likely to affect miRNA expression. There may, of course, be other confounders we were unable to control for in the present study. For example, changes in the cellular composition of the islets (i.e. alpha/beta cell ratios) cannot be excluded, though this would seem to be unlikely. Future studies, involving even larger islet cohorts, seem very likely to find further aberrantly expressed miRNAs in islets from individuals with type 2 diabetes; however, our study provides proof-of-principle that miRNA expression profiling in islets from individuals with and without diabetes can identify miRNAs with a causal role in beta cell dysfunction.

Using direct functional assays in both primary rodent islets and a rat insulinoma-derived cell line, we provide evidence here that increases in miR-187 affect glucose- and, to a lesser extent, depolarisation-induced insulin secretion, without evident effects on cell viability or apoptotic index. Importantly, the dramatic inhibition of GSIS elicited by miR-187 was similar in extent to what we observed after transfection with a mimic of miR-375 (data not shown). MiR-375 has a well-established role in the control of insulin release [19] and our results suggest that miR-187 may play an equally important role in this process after its induction in the beta cell of individuals with type 2 diabetes. Given the much greater impact of miR-187 overexpression on glucose- compared with KCl-stimulated secretion, it would appear that miR-187 acts chiefly on events upstream of membrane depolarisation, potentially impairing glucose metabolism or increases in free cytosolic Ca^{2+} [20]. Detailed future studies will be needed to investigate these possibilities. Likewise, analysing the effects of overexpressing miR-187 in primary human islets and in response to additional physiological secretagogues (such as glucagon-like peptide-1, acetylcholine and amino acids) also represent important future experiments.

By providing one possible mechanism through which miR-187 may act we show here that *HIPK3* is a target for this miRNA in the beta cell. Importantly, HIPK3 has recently been shown to be required for the normal stimulation of insulin secretion by glucose. Thus, *Hipk3*$^{-/-}$ mice are glucose intolerant and show depressed islet levels of two key mediators of glucose responsiveness: pancreatic duodenum homeobox-1 (PDX1) and phosphorylated glycogen synthase kinase-3 β (GSK3β) [15]. Although we observed only small decreases in luciferase activity and *Hipk3* transcript levels on miR-187 overexpression, this is consistent with a role for miRNAs in fine-tuning gene expression [21, 22]. Additionally, given that in mouse islets a modest 46% knockdown of HIPK3 reduces insulin secretion at 20 mmol/l to 54% of that seen in control cells [15], it seems plausible that small decreases in *Hipk3* expression may have a significant impact on GSIS. We emphasise that while the decrease in *Hipk3* mRNA levels in the presence of miR-187 may conceivably be compounded by a decrease in translational efficiency, recent studies suggest that the actions of miRNAs are largely (~84%) mediated by RNA degradation [23]. Nonetheless, studies are needed to explore this possibility.

Aberrant miR-187 expression has, to our knowledge, not been reported in any previous study examining miRNA expression in islets from animal models of type 2 diabetes, nor in cell-line models using culture conditions that mimic those found in an individual with diabetes. While evidence of deregulated miR-187 expression in these experiments might strengthen and support our data it is perhaps not surprising, given the difficulties in modelling polygenic diseases in controlled animal and cell culture systems, that no such result has been reported previously. Indeed, the molecular mechanisms through which miR-187 expression is increased in islets from donors with type 2 diabetes remain obscure.

It is interesting that two recent studies detailing the human islet miRNome in individuals without diabetes report either low miR-187 expression [24] or a failure to detect miR-187 in the majority of samples studied [25] (it is assumed that ΔC_t values and normalised RNA-Seq read counts can be used as appropriate proxies for absolute miRNA levels). We also find that miR-187 is relatively weakly expressed in individuals without diabetes. However, the five- to sevenfold increase in miR-187 expression in islets from individuals with type 2 diabetes means its levels in this pathophysiological state are comparable with, or greater than, levels of several other miRNAs with known roles in beta cell function (miR-29b [26], miR-9 [27] and miR-96 [28]). Perhaps, akin to what has been assumed to account for the relatively low steady-state expression of other regulatory genes (such as transcription factors) [29], a low expression of miR-187 is needed to provide a built-in fail-safe mechanism, controlling its persistence and thus preventing aberrant beta cell function.

We are aware of only one other publication comparing miRNA expression in human islets from individuals with and without glucose intolerance. While using a far smaller number of samples than the present study ($n=9$ islets from individuals without diabetes, $n=6$ from individuals with an $HbA_{1c} \geq 6.1$), and also examining the expression of only a small number of miRNAs (not including miR-187), this earlier study provided some evidence for abnormal miRNA expression in islets from individuals with type 2 diabetes [30]. The results of our larger study highlight that aberrantly expressed miRNAs may be causally involved in human islet dysfunction during type 2 diabetes. Future studies identifying further dysregulated miRNAs may discover novel pathways involved in islet dysfunction that could provide novel therapeutic targets for diabetes treatment.

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

Contribution statement JML designed and conducted experiments, analysed data and wrote the manuscript. GdSX and HRD designed, conducted and analysed data from experiments. GAR contributed to the study design, data analysis and writing the manuscript. LWH was involved in the study design, writing the manuscript and managed the project. All authors participated in data interpretation, revision of the article and approved the final version of the manuscript.

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