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ORIGINAL ARTICLE

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Pharmacological activation of insulin-degrading enzyme improves insulin secretion and glucose tolerance in diet-induced obese mice

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Abstract

Aim: To investigate the use of synthetic preimplantation factor (sPIF) as a potential therapeutic tool for improving glucose-stimulated insulin secretion (GSIS), glucose tolerance and insulin sensitivity in the setting of diabetes.

Materials and Methods: We used a preclinical murine model of type 2 diabetes (T2D) induced by high-fat diet (HFD) feeding for 12 weeks. Saline or sPIF (1 mg/kg/day) was administered to mice by subcutaneously implanted osmotic mini-pumps for 25 days. Glucose tolerance, circulating insulin and C-peptide levels, and GSIS were assessed. In addition, β-cells (Min-6) were used to test the effects of sPIF on GSIS and insulin-degrading enzyme (IDE) activity in vitro. The effect of sPIF on GSIS was also tested in human islets.

Results: GSIS was enhanced 2-fold by sPIF in human islets ex vivo. Furthermore, continuous administration of sPIF to HFD mice increased circulating levels of insulin and improved glucose tolerance, independently of hepatic insulin clearance. Of note, islets isolated from mice treated with sPIF exhibited restored β-cell function. Finally, genetic (shRNA-IDE) or pharmacological (6bK) inactivation of IDE in Min-6 abolished

Germán Perdomo and Beatriz Merino: both authors contributed equally.

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sPIF-mediated effects on GSIS, showing that both the protein and its protease activity are required for its action.

Conclusions: We conclude that sPIF is a promising secretagogue for the treatment of T2D.

KEYWORDS
antidiabetic drug, beta cell function, drug mechanism, insulin secretagogue, insulin secretion

1 | INTRODUCTION

Preimplantation factor (PIF) is a 15-amino acid peptide (MVRIKP GSANKPSDD) that is expressed by both the mammalian embryo and its placenta. It is found in the early stages of pregnancy and remains in the mother's bloodstream throughout the whole course of a viable pregnancy.[1–5] Recent research has shown that PIF supports embryo development in the early stages of pregnancy by having a variety of functions, including regulating systemic immunity, adhesion, remodelling, apoptosis and trophoblast invasion.[4,6,7] Interestingly, synthetic PIF (sPIF) mimics native PIF action both in vitro and in vivo (reviewed in[7]).

In clinical settings, sPIF administration is safe and well tolerated.[8] The immune-modulatory properties of sPIF allow for its therapeutic use in the treatment of human diseases. In this context, sPIF has displayed a prophylactic role in type 1 diabetes (T1D)[9] and cardiovascular disease[10] in preclinical models.

Insulin-degrading enzyme (IDE) is a widely expressed and well-conserved Zn^{2+} neutral metallo-endopeptidase.[11,12] IDE has a strong affinity for insulin ($K_m \sim 0.1$ μM), and degrades the hormone into several fragments.[11,12]

PIF predominantly targets IDE, activating its proteolytic function, as determined by Paidas et al.[13] The core sequence R3I4K5P6 of the PIF peptide regulates IDE function, and the particular I4 and P6 (MVR**I**K**P**GSANKPSDD) amino acids are necessary for hydrophobic interactions with IDE.[13] Intriguingly, the PIF-IDE complex forms a high affinity bond when insulin is present, indicating that PIF and insulin are sterically in direct competition.[14] PIF may stabilize IDE in the open conformation and increases substrate accessibility to its active pocket, while the specifics of this interaction are still unclear.[14]

In the last few years, it has been suggested that IDE may have a role in the pathophysiology of type 2 diabetes (T2D). Thus, genetic variants within or close to the *Ide* locus have been associated with a higher risk of T2D in different ethnic groups.[15-19] Circulating blood IDE levels were elevated in T2D patients and in subjects with metabolic syndrome.[20,21] African Americans were shown to have greater plasma insulin levels and decreased hepatic IDE activity.[22] Likewise, individuals with T2D had lower hepatic IDE expression.[23] Additionally, Fernandez-Díaz et al. showed that IDE, which is expressed in both α- and β-cells, is decreased in β-cells from T2D patients receiving oral hypoglycaemic agents.[24]

Given that IDE may play an important role in T2D, the development of IDE inhibitors has attracted growing attention as a pharmacological approach for people with diabetes, with contentious outcomes regarding their use as modulators of insulin clearance and glucose tolerance (reviewed in[11]). However, we reported that overexpressing IDE in the liver of diet-induced obese mice improved insulin sensitivity and glucose homeostasis.[25] These findings raise the possibility that developing IDE activators, as opposed to IDE inhibitors, may be a more effective pharmacological strategy for the treatment of T2D. Using human islets, Min-6 cells, and a diet-induced obese mouse model of T2D, we have searched for the potential therapeutic use of sPIF to enhance glucose-stimulated insulin secretion (GSIS), glucose tolerance and insulin sensitivity.

2 | MATERIALS AND METHODS

2.1 | Human islets

Experiments involving human islets from Montpellier Hospital (LTCD) were performed in agreement with the local ethic committee (Biological Ressources Center, Collection IRB 5 'Human Islets of Langerhans', BiobanK no. BB-0033-00031, CHU, Montpellier), the institutional ethical committee of the French Agence de la Biomédecine (ABM no. PFS 13-008) and the French Ministry of Research (DC-2011-1401 and AC-2017-3039). Pancreas was harvested from a brain-dead non-diabetic donor (donor profile: male, 37 years old, 181 cm per 85 kg, body mass index 25.9 kg/m^2, death by aneurysm rupture. Smoking and epileptic patient treated with depakin). See the supporting information (Supplementary appendix) for organ donation, informed consent and human islets isolation.

2.2 | Animal experimentation

Four- to five-week-old male C57BL/6J mice were purchased from Envigo (Indiana, EEUU). The mice were housed in ventilated cages under a 12-hour:12-hour light–dark cycle with water available ad libitum, at the animal facility of the University of Valladolid (UVa). The Animal Care and Use Committee of the UVa approved all experiments (protocol #8608731). Mice were fed a standard rodent diet (SD; Research Diets # D12450J, 20% protein, 70% carbohydrates, 10% fat) or a high-fat diet (HFD; Research Diets # D12492, 20% protein, 20% carbohydrates, 60% fat) for 12 weeks. Randomization of mice was performed before the treatment with saline or sPIF using an Excel

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spreadsheet. Then mini-pumps (Alzet, USA) containing saline or sPIF were subcutaneously implanted in the upper back of the mice. The rate of delivery was 1 mg/kg/day. The pumps remained implanted for 4 weeks; during that time the animals were fed their corresponding food (SD or HFD). Afterwards, mice were euthanized and blood and tissues (pancreas and pancreatic islets) were collected for plasma biochemistry analyses, immunohistochemistry and GSIS assessment.

2.3 | Glucose tolerance test and plasma determination of insulin and C-peptide

To evaluate alterations in glucose homeostasis, we performed an intraperitoneal glucose tolerance test (IPGTT). Mice were fasted for 16 hours and then injected with 2 g glucose/kg body weight, as previously described.[25] Blood samples were collected from the tail vein using capillary tubes precoated with potassium-EDTA (Sarstedt, Germany), and plasma was obtained by centrifugation at 3300 $\times g$ for 15 minutes at 4°C. Insulin and C-peptide levels were determined by ELISA kit (Mercodia, Sweden), following the manufacturer's instructions. To determine hepatic insulin clearance, the molar ratio C-peptide/insulin was calculated from fasting insulin and C-peptide levels, as previously described.[25] The homeostatic model assessment (HOMA) index was calculated using fasting insulin and glucose concentrations, according to.[26–29]

Hepatic plasma insulin clearance was assessed in two different ways: (1) as the steady-state C-peptide/insulin molar ratio, previously described by our group.[25] To achieve the steady-state, mice were fasted from 06:00 PM until 11:00 AM the next morning; or (2) during the IPGTT, as described by Kurauti et al.,[30,31] with some modifications. Briefly, mice were fasted overnight followed by an intraperitoneal injection of glucose, and blood samples were drawn from the tail vein at 0, 5, 15 and 30 minutes. Plasma insulin and C-peptide levels were assessed by ELISA (Mercodia). Then the C-peptide/insulin molar ratio was calculated for each time point, and the area under the curve (AUC) at times 0-30 minutes (AUC_{0-30}) was calculated as a measure of insulin clearance.

2.4 | Pancreas histomorphometry

Pancreas histomorphometry (β-cell mass, islet number and islet size) was performed as previously described in a double-blind procedure.[32,33]

2.5 | Islet isolation, ex vivo GSIS and determination of intracellular insulin content

Mouse islets isolation, assessment of GSIS and quantification of intracellular insulin content were performed as previously described.[32,33] Detailed protocols are available in the supporting information (supplementary appendix).

2.6 | Cell culture and in vitro GSIS

Min-6 cells were grown in Dulbecco's modified eagle medium (4.5 g/L glucose) supplemented with 15% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate and 50 μM β-mercaptoethanol at 37°C and 5% CO_2 in a humidified atmosphere to 80% confluence. Detailed protocols of IDE knockdown in Min-6 by shRNA-IDE and assessment of GSIS in Min-6 are described in the supporting information (supplementary appendix).

2.7 | Western blot analyses

Western blot analyses on Min-6 cells and islets were performed as described.[32] Detailed protocols are available in the supporting information (supplementary appendix).

2.8 | IDE activity

IDE activity was assessed with the fluorometric SensoLyte 520 IDE activity assay kit (AnaSpec, Inc., USA), as previously described.[34] A detailed protocol is available in the supporting information (supplementary appendix).

2.9 | Synthesis of sPIF

sPIF peptide was synthesized manually using a Fmoc/tBu solid-phase strategy on a 2-chloro trityl chloride resin (CTC) at the Synthesis of Peptides Unit (U3; https://www.nanbiosis.es/portfolio/u3-synthesis-of-peptides-unit/) of the ICTS NANBIOSIS at the IQAC. All Fmoc-protected amino acids (Fmoc–aa-OH) were supplied by Iris Biotech (Germany). The C-terminal residue was introduced onto the CTC resin by the addition of the corresponding Fmco–aa-OH (1 mmol) and diisopropylethylamine (8.0 mmol) dissolved in anhydrous dichloromethane (DCM) on the solid support. After 1.5 hours, MeOH was added (0.8 mL/g resin) and the mixture was left to react for 30 minutes. Then the mixture was filtered and the resin was washed with N,N-dimethylformamide (DMF) and DCM. Both peptides were elongated by a sequence of Fmoc elimination and Fmoc-protected amino acid coupling cycles, with washings with DMF between both processes. Treatments with piperidine:DMF solution (2:8, v/v) were used to eliminate the Fmoc group and the protected amino acid was introduced onto the resin by reaction with a mixture of Fmoc-aa-OH (3 equiv.), N,N′-diisopropylcarbodiimide (3 equiv., DIC) and ethyl cyano(hydroxyimino)acetate (3 equiv., oxyme pure) in DMF. Once the elongation process was finished, final peptidyl resins were hydrolysed with TFA:H_2O:triisopropylsilane (95:2.5:2.5, v/v/v) and precipitated in diethyl ether. The final peptide crude solution was lyophilized. sPIF was purified by semipreparative high-performance liquid chromatography (HPLC) semiprep and analysed by HPLC and HPLC-mass spectrometry (MS).

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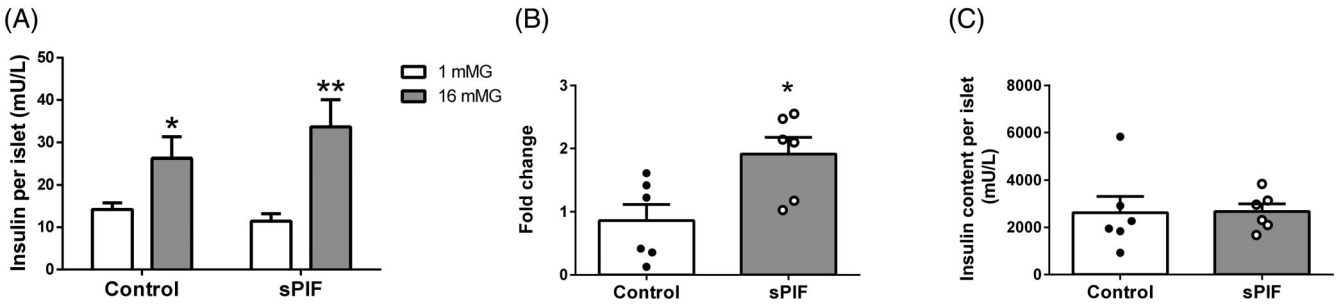

FIGURE 1 sPIF increases GSIS in human islets. A, GSIS of human islets untreated or treated with sPIF (50 nM) for 16 hours; $N = 6$ groups of 10 islets; 60 islets per GSIS condition. Data are reported as means ± SEM. $^{*}P < .05$ or $^{**}P < .01$ versus 1 mM glucose by one-way ANOVA. B, Fold change from insulin secretion of panel A. Data are reported as means ± SEM; $N = 6$ groups of 10 islets; 60 islets per GSIS condition. $^{*}P < .05$ versus control by unpaired Student's *t*-test. C, Intracellular insulin content of human islets measured after GSIS. Data are reported as means ± SEM; $N = 6$ per condition. ANOVA, analysis of variance; GSIS, glucose-stimulated insulin secretion; sPIF, synthetic preimplantation factor.

sPIF 15: 128.1 mg. Chemical purity (estimated by ultraviolet at 220 nm): 99%. MS: calculated mass for C67H115N21O23S 1614.84; found: 1615.8 [M + H] +; 808.2 [M + 2H]2+; 539.4 [M + 3H]3+; 404.9 [M + 4H]4+ (Figure **S1**A,B).

2.10 | Statistical analysis

Statistical analysis was performed using Prism v. 6.0 (GraphPad Software, Inc., San Diego, CA). The normality of the distribution of data was checked with the Kolmogorov–Smirnov test. For parametric data, comparisons between the two groups were assessed using the paired or unpaired Student's *t*-test. Comparisons between more than two groups were performed using one-way or two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. For non-parametric data, comparisons between the two groups were assessed using the Mann–Whitney U test. Data are presented as the means ± SEM. Differences were considered significant at *P* less than .05.

3 | RESULTS

3.1 | sPIF enhances GSIS in human islets ex vivo

We have previously shown that IDE deficiency in β-cells leads to dysregulation of GSIS.[32] sPIF has been described as an activator of the IDE's proteolytic activity.[14] We hypothesized that sPIF-mediated activation of IDE may be beneficial for the upregulation of glucose-mediated insulin secretion in β-cells. To clarify this, we treated isolated islets from one non-diabetic human donor with sPIF (50 nM) for 16 hours, followed by GSIS. GSIS was enhanced 2-fold by sPIF in human islets (Figure 1A,B). Furthermore, this effect was not related to changes in intracellular insulin content (Figure 1C).

3.2 | sPIF-mediated activation of IDE increases GSIS in mouse pancreatic β-cells

To investigate the effect of sPIF on glucose-dependent insulin secretion in β-cells, we performed GSIS and assessed IDE protease activity in Min-6 cells in the absence or the presence of sPIF (50 nM) for 16 hours. As expected, high glucose increased insulin secretion by ∼ 7-fold (Figure 2A, B). Likewise, sPIF augmented insulin secretion by ∼13-fold compared with control cells (Figure 2A, B). These results suggest that enhanced GSIS observed in human islets treated with sPIF is most probably attributable to improved function of the pancreatic β-cells.

As shown in Figure 2C, sPIF treatment (50 nM, 16 hours) increased IDE activity by 32% compared with untreated cells. Importantly, sPIF treatment did not alter IDE protein levels (Figure **S2**). The augmented IDE activity parallels the enhanced capacity for insulin secretion in β-cells (Figure 2A–C). To gain insights into the mechanism(s) by which sPIF augmented insulin secretion in β-cells, we genetically lowered IDE expression levels in Min-6 cells using shRNA-IDE. This approach resulted in a 70% reduction in IDE levels (Figure 2D,E) in parallel with a 30% decrease in its activity (Figure 2C), which was associated with a 2-fold reduction in GSIS capacity compared with control cells (Figure 2A,B). Interestingly, sPIF treatment in shRNA-IDE Min-6 cells did not restore GSIS function (Figure 2A–C). Taken together, these results show that IDE is necessary for sPIF-mediated effects on GSIS in Min-6 cells.

To further decipher the mechanism(s) by which sPIF improves insulin secretion in β-cells, we assessed GSIS in Min-6 cells in the absence or the presence of 6bK (10 μM, 30 minutes), a highly specific inhibitor of IDE activity.[29,35] As shown in Figure 3A,B, 6bK treatment did not affect GSIS. However, sPIF treatment (50 nM, 16 hours) did not increase insulin secretion in β-cells when cells were preincubated with 6bK (Figure 3A,B). Taken together, these results show that the mechanism of action of sPIF on GSIS requires intact IDE activity in β-cells.

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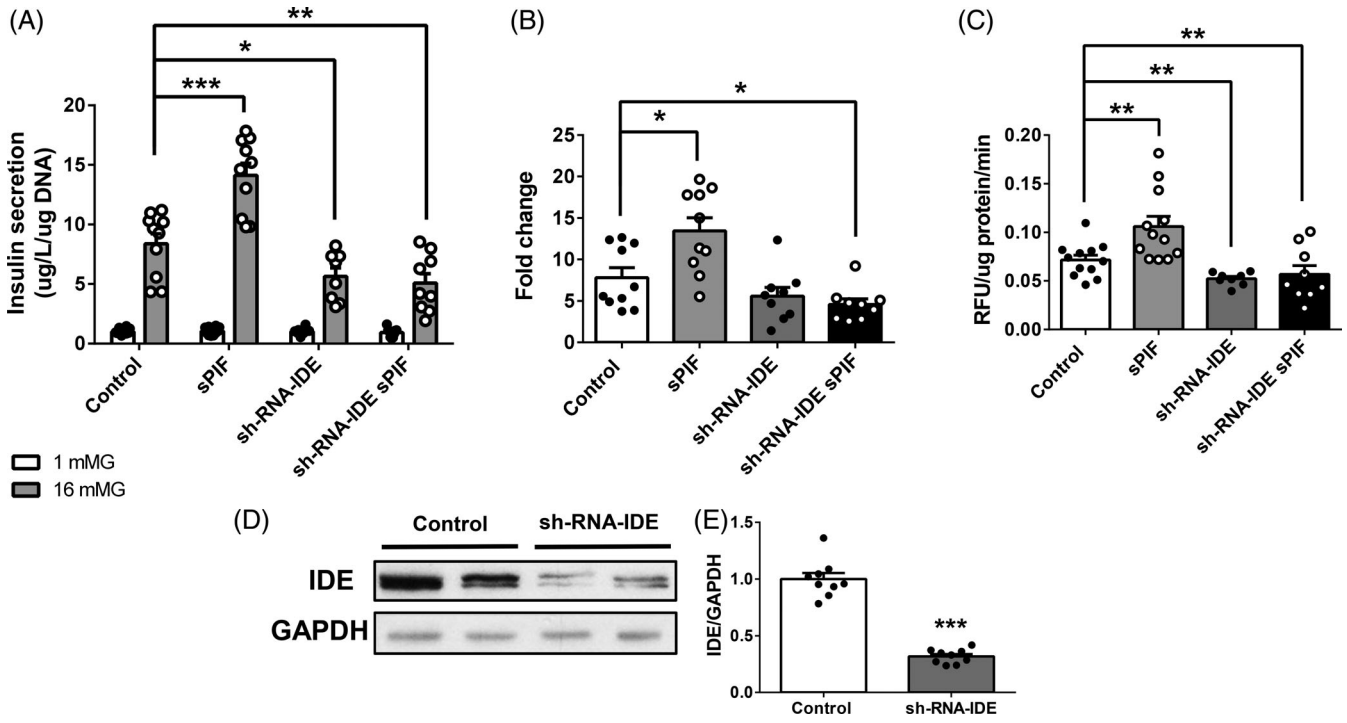

FIGURE 2 sPIF increases β-cell function and IDE activity in Min-6 cells. A, Effects of sPIF on GSIS in β-cells in the absence or presence of 50 nM sPIF for 16 hours. The experiments were carried out in control cells (Min-6) or in Min-6 cells transduced with lentiviruses containing shRNA-IDE (shRNA-IDE). Data are reported as means ± SEM; $N = 3$ independent experiments (in triplicate). $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$ by two-way ANOVA. B, Fold change from insulin secretion of panel A. Data are reported as means ± SEM; $N = 3$ independent experiments (in triplicate). $^{*}P < .05$ by one-way ANOVA. C, Effects of sPIF on IDE activity in Min-6 cells in the presence or absence of sPIF 50 nM for 16 hours. The experiments were carried out in control cells (Min-6) or in Min-6 cells with genetic ablation of IDE (shRNA-IDE). Data are reported as means ± SEM; $N = 3$ independent experiments (9-10 replicates). $^{**}P < .01$ by one-way ANOVA. D, Expression levels of IDE by Western blot in Min-6 cells with shRNA-IDE versus control cells. Representative image of control cells (Min-6) and shRNA-IDE cells. GAPDH expression was determined to ensure similar protein loading. E, Western blot quantification of panel D. Data are reported as means ± SEM; $N = 3$ independent experiments (in triplicate). $^{***}P < .01$ versus control by unpaired Student's *t*-test. ANOVA, analysis of variance; GSIS, glucose-stimulated insulin secretion; IDE, insulin-degrading enzyme; RFU, relative fluorescence units; sPIF, synthetic preimplantation factor.

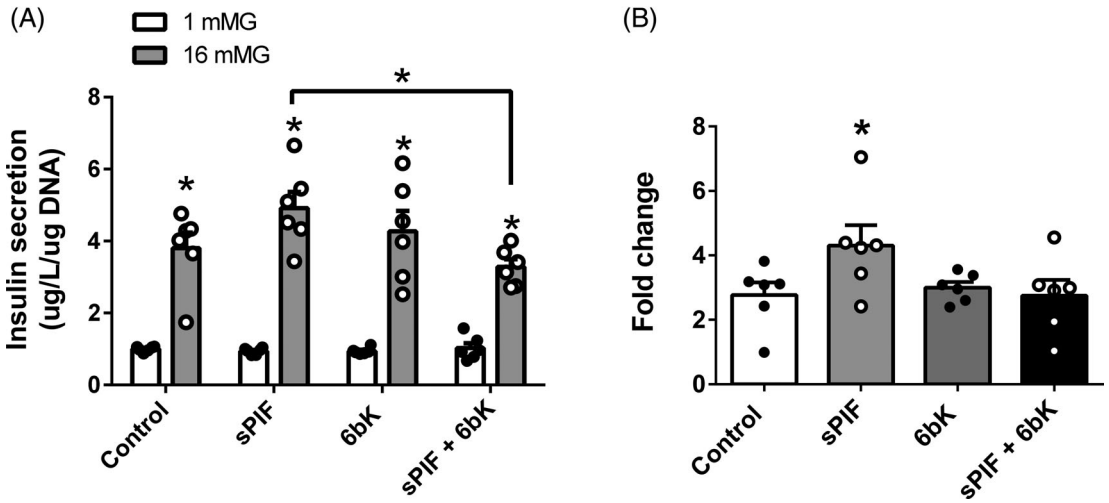

FIGURE 3 The IDE-specific inhibitor 6bK reverses sPIF-mediated effect on GSIS in β-cells. A, Effects of 6bK on insulin secretion in β-cells in the absence or presence of 50 nM sPIF for 16 hours. Min-6 cells were preincubated with 10 μM 6bK inhibitor for 30 minutes before assessment of insulin secretion. Data are reported as means ± SEM; $N = 6$ per condition. $^{*}P < .05$ by two-way ANOVA. B, Fold change from insulin secretion. Data are reported as means ± SEM; $N = 6$ per condition. $^{*}P < .05$ versus control by one-way ANOVA. ANOVA, analysis of variance; GSIS, glucose-stimulated insulin secretion; IDE, insulin-degrading enzyme; sPIF, synthetic preimplantation factor.

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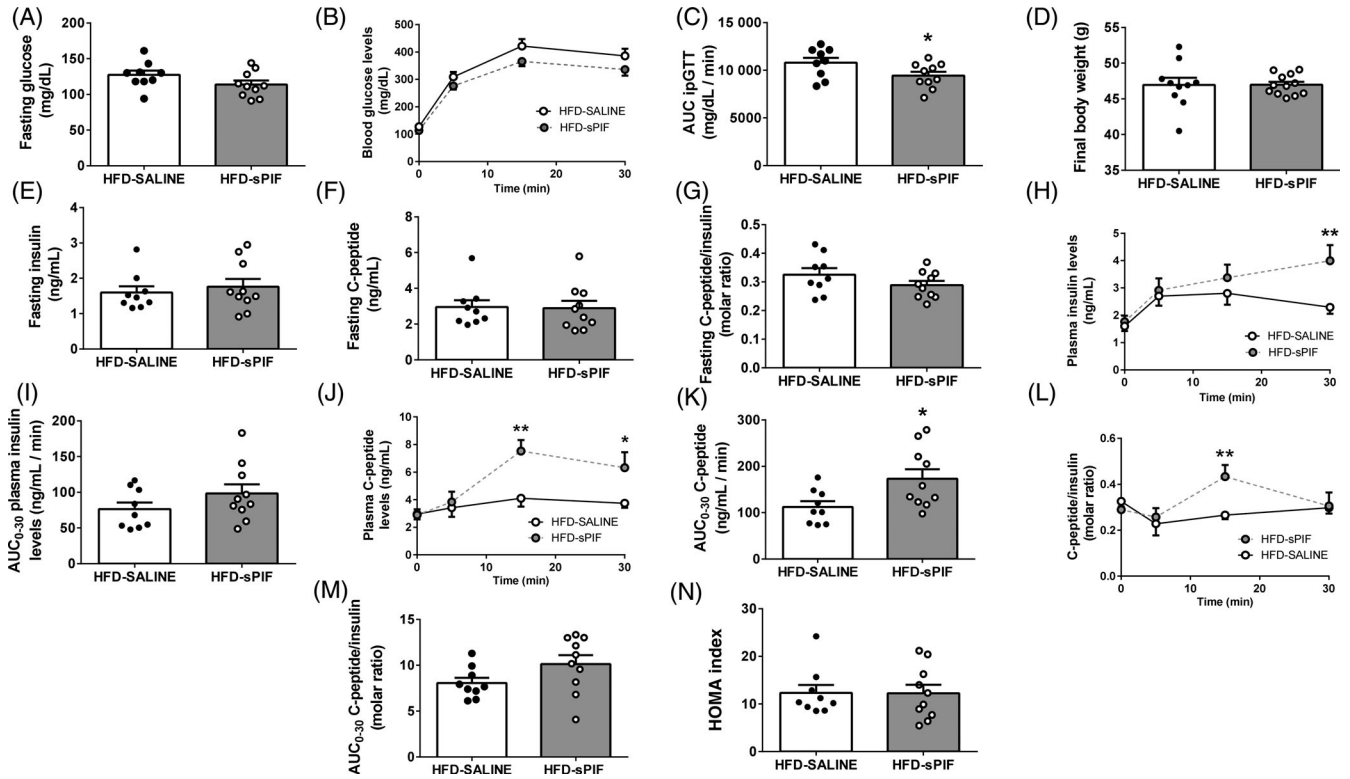

FIGURE 4 Continuous administration of sPIF improves glucose tolerance and circulating insulin levels in diet-induced obese mice. A, Fasting glucose; B, Intraperitoneal glucose tolerance test (ipGTT); C, Area under the curve (AUC) of ipGTT; D, Body weight; E, Fasting insulin levels; F, Fasting C-peptide levels; G, Steady-state C-peptide/insulin molar ratio; H, Plasma insulin levels during the ipGTT; I, AUC of plasma insulin levels during the ipGTT; J, Plasma C-peptide levels during the ipGTT; K, AUC of C-peptide levels during the ipGTT; L, C-peptide/insulin molar ratio during the ipGTT; M, AUC of C-peptide/insulin molar ratio during the ipGTT; N, HOMA index. Data are represented as means ± SEM. HFD-SALINE ($n = 9$ mice); HFD-sPIF ($n = 10$) mice. *$P < .05$; **$P < .01$ by unpaired Student's t-test. In the case of panel E, data were analysed by Mann–Whitney U test. HOMA, homeostatic model assessment; sPIF, synthetic preimplantation factor.

3.3 | sPIF improves circulating insulin levels and glucose tolerance in diet-induced obese mice

To investigate the effect of sPIF as a therapeutic treatment in the setting of obesity and T2D, C57BL/6J mice were fed a HFD. After 12 weeks, mice showed obesity, hyperglycaemia, glucose intolerance, insulin resistance and hyperinsulinaemia (Figure S3). Then the animals were randomized into two groups and implanted with a subcutaneous mini-pump containing saline (HFD-SALINE) or sPIF (HFD-sPIF) at a 1 mg/kg/day dose, while maintaining HFD feeding. After 21 days of treatment, HFD-sPIF–treated mice showed a slight improvement in fasting glycaemia (Figure 4A) and exhibited significant improvement in glucose tolerance compared with saline-treated mice (Figure 4B,C). These improvements in glucose homeostasis were not associated with lower body weight (Figure 4D). On the other hand, fasting plasma insulin and C-peptide levels remained unchanged in the HFD-sPIF group (Figure 4E,F). Likewise, the steady-state C-peptide/insulin molar ratio remained unchanged between the HFD-SALINE and HFD-sPIF groups (Figure 4G). However, after a glucose challenge, both insulin (Figure 4H,I) and C-peptide (Figure 4J,K) levels increased significantly. These improvements in insulin homeostasis were not associated with changes in the insulin clearance during the IPGTT (Figure 4L,M), nor with changes in the HOMA index, a surrogate marker of insulin sensitivity (Figure 4N). These findings suggest that the increased circulating insulin levels seen in sPIF-treated mice are most probably attributable to improved β-cell function and/or β-cell mass, rather than changes in insulin clearance.

3.4 | sPIF-treated obese mice exhibited an enhanced capacity for insulin secretion but unchanged β-cells mass

Next, we performed histomorphometry analyses of pancreas dissected from HFD-SALINE– and HFD-sPIF–treated mice. The weight of the pancreas, β-cell area, β-cell mass, islets number and islets size remained unchanged between untreated and sPIF-treated mice (Figure S4). These results support the notion that the effect of sPIF on circulating insulin levels would be related to β-cell function. Thus, we assessed GSIS in isolated islets from SD-SALINE–, HFD-SALINE– and HFD-sPIF–treated mice. As expected, high glucose induced 1.5-fold insulin secretion in islets isolated from control mice (SD-SALINE) (Figure 5A,B). By contrast, islets isolated from mice fed a HFD (HFD-SALINE) lost the ability to secrete insulin in response to high

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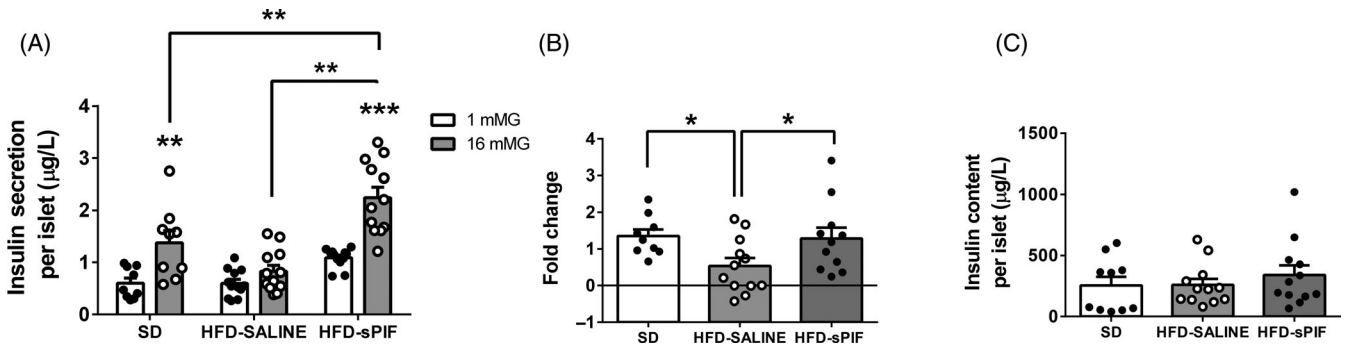

FIGURE 5 Continuous administration of sPIF recovers β-cell function in islets isolated from diet-induced obese mice. A, Mice were fed a SD or a HFD, and saline or sPIF was continuously administered as described in the Materials and Methods section. Afterwards, islets were isolated and GSIS was assessed. Data are reported as means ± SEM. SD ($n = 2$ mice), 9 islet groups; 5 islets per group. HFD-SALINE (n = 3 mice), 12 islet groups; 5 islets per group. HFD-sPIF ($n = 3$ mice), 12 islet groups; 5 islets per group. $^{**}P < .01$; $^{***}P < .001$ by two-way ANOVA. B, Fold change from insulin secretion of panel A. Data are reported as means ± SEM. SD ($n = 2$ mice), 9 islet groups; 5 islets per group. HFD-SALINE (n = 3 mice), 12 islet groups; 5 islets per group. HFD-sPIF ($n = 3$ mice), 12 islet groups; 5 islets per group. $^{*}P < .05$ by one-way ANOVA. C, Intracellular insulin content of mice islets measured after GSIS. Data are reported as means ± SEM. SD ($n = 2$ mice), 9 islet groups; 5 islets per group. HFD-SALINE ($n = 3$ mice), 12 islet groups; 5 islets per group. HFD-sPIF ($n = 3$ mice), 12 islet groups; 5 islets per group. ANOVA, analysis of variance; GSIS, glucose-stimulated insulin secretion; HFD, high-fat diet; SD, standard diet; sPIF, synthetic preimplantation factor.

glucose levels (Figure 5A,B). Of note, islets isolated from sPIF-treated mice (HFD-sPIF) exhibited 1.5-fold induction of glucose-stimulated insulin secretion (Figure 5A,B). Finally, these changes in the capacity to secrete insulin were not related to the insulin content in the islets (Figure 5C). Taken together, these results show that prolonged sPIF treatment restores the reduced insulin-secreting capacity of pancreatic islets in obese mice.

To further investigate the mechanisms by which sPIF improves insulin secretion, we analysed the expression levels of some genes involved in the regulation of insulin secretion in β-cells. To this end, we used Min-6 cells preincubated in the absence or presence of sPIF for 16 hours. We performed qPCR for the genes coding for the subunits KIR and SUR of the KATP channel (KCNJ11 and ABCC8), a transcriptional factor involved in the regulation of β-cell identity (PDX-1), the insulin mouse genes (INS1 and INS2) and the glucose transporter GLUT2 (GLUT2). As shown in Figure S5, the expression of the subunits KIR and SUR was upregulated in response to sPIF treatment, whereas the expression of PDX-1, INS1, INS2 and GLUT-2 remained unchanged by sPIF treatment.

4 | DISCUSSION

The last decade has awoken considerable interest in IDE as a therapeutic target for T2D, putting the focus on the development of pharmacological inhibitors. This rationale was grounded in the observation that pancellular deletion of IDE in mice (IDE-KO) resulted in hyperinsulinaemia.[36] However, the development of IDE tissue-specific knockout mouse models in the liver and pancreatic β and α-cells (L-IDE-KO, B-IDE-KO and A-IDE-KO, respectively) has provided important knowledge regarding the proteolytic and non-proteolytic functions of IDE.[12,32,33] These models showed that IDE deficiency was associated with metabolic phenotypes of T2D (i.e. glucose intolerance, hyperinsulinaemia and hyperglucagonaemia), questioning the rationale for the use of IDE inhibitors in T2D.

In humans, some studies have shown that impaired release of insulin was linked to genetic variations around the *Hhex/Ide* chromosomal locus.[37,38] Steneberg et al. reported lower IDE protein levels in islets from patients with T2D compared with subjects without diabetes.[39] Furthermore, Fernández-Díaz et al. showed that IDE protein levels were lower in the β-cells of diabetes patients than in subjects without diabetes.[24] On the other hand, the Goto-Kakizaki rat, a preclinical model of diabetes, harbours missense mutations in the *Ide* gene.[40] In mice, loss of IDE in β-cells (B-IDE-KO) alters the expression of key genes necessary for its physiological function, leading to the secretion of immature insulin granules and constitutive insulin secretion, independently of glucose levels.[32] Conversely, Steneberg et al. reported insufficient insulin secretion from islets isolated from IDE-KO mice.[39] These findings highlight that regulation of IDE levels in β-cells modulates insulin secretion. Nonetheless, a constraint of these models is that genetic manipulations of IDE have been limited to its deletion, which inhibits its activity but also diminishes the protein levels.

Studies using pharmacological inhibitors would enable the proteolytic activity of IDE to be examined while leaving the protein structure unaltered, which could provide information on how IDE activity affects the regulation of insulin secretion from β-cells and its impact on glucose homeostasis. However, the use of IDE inhibitors as regulators of insulin proteostasis and glucose homeostasis have yielded controversial results (reviewed in[11,41]). For example, human and rat islets treated with **NTE-2**, a specific inhibitor of IDE, were found to have detrimental effects on GSIS.[24,32,42] **6bK**, when tested in mice fed a HFD, increased plasma insulin levels, but also amylin and glucagon levels.[35] On the other hand, **ADT21**, an IDE inhibitor derived from the protein sequence of varicella-zoster, showed delayed onset of diabetes in NOD mice (a preclinical model of T1D) and improved oral glucose tolerance in diet-induced obese mice.[43]

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Less attention has been paid to the development of IDE activators. We have shown the principle-of-proof that hepatic gain-of-function of IDE is beneficial for improving glucose tolerance and insulin sensitivity in diet-induced obese mice.[25] These findings spurred the hypothesis that upregulation of IDE activity might be used as a strategy to improve the glucose-secreting capacity of β-cells in the setting of T2D.

Because of IDE's ability to clear Aβ peptide,[44] the enzymatic activation of IDE has been traditionally explored as a potential treatment for Alzheimer's disease (AD). Compounds such as sumarin,[45] **Ia1** and **Ia2** (aka LDN-1487 and LDN-1844),[46] cell-permeable small molecules[47] or other polyaromatic compounds,[48] were identified as IDE activators in vitro, but none of them have been shown to have beneficial or detrimental effects on insulin secretion. Interestingly, the indole-based compound **1** (aka BDM35899), an IDE activator for Aβ and insulin hydrolysis, decreases GSIS in pancreatic β-cells.[41] Docking studies showed that this compound binds to the polyanion binding site of IDE, but not to its catalytic site.[41] Conversely, sPIF competes with insulin by the catalytic site.[14] These differences in the binding mechanisms between BDM35899 and sPIF on IDE may be critical for explaining the opposing effects of BDM35899 and sPIF on glucose-stimulated insulin secretion in β-cells. Because both BDM35899 and sPIF promote the degradation of insulin and Aβ, the mechanisms by which these compounds enhance the proteolytic activity of IDE may be of relevance for their effectiveness as potential drugs for the treatment of AD or diabetes.

In this work, we investigated the potential impact of sPIF-mediated activation of IDE on insulin clearance in the setting of obesity. To this end, we used the insulin clearance rate (measured as the steady-state C-peptide/insulin molar ratio in fasting or during a glucose challenge) in diet-induced obese mice. No differences in the rate of insulin extraction were observed in response to sPIF administration. These data agree with our previous studies, where neither the loss (L-IDE-KO mouse) nor the gain (adenoviral delivery) of IDE function altered the insulin clearance rate.[25,49] However, the role of IDE on hepatic insulin clearance remains controversial. Thus, Borges et al. reported that liver-specific knockout mice fed a chow diet showed reduced postprandial insulin clearance.[50] On the other hand, in the current study, we have used the C-peptide: insulin molar ratio, as well as the ratio between the incremental AUCs of the same peptides, after administration of an intraperitoneal bolus of glucose. These simple calculations have been extensively used both in the fasting state and after glucose challenges.[51] A limitation of this method was pointed out by Polonsky and Rubenstein.[52] Thus, insulin and C-peptide have very different plasma half-lifes (4 vs. 30 minutes, respectively), and the C-peptide: insulin molar ratio in the blood depends not only on the release rates of these peptides from the pancreatic islets, but also upon their individual disappearance kinetics. Finally, the gold standard to measure hepatic insulin clearance is the use of hyperinsulinaemic–euglycaemic clamp analysis.[53] Further research using this technique in mice is warranted to clarify the role of sPIF-mediated activation of IDE on hepatic insulin clearance.

PIF exerts an immune modulatory effect on human endometrium, promoting immune tolerance to the embryo during pregnancy.[54] Furthermore, several studies have shown the positive effect of sPIF on autoimmune diseases, neurodegenerative disease and pregnancy disorders.[7] It should be noted that sPIF reduces neuroinflammation and neurodegeration in different animal models[55–57] and prevents inflammation-mediated foetal loss.[58,59] Low-grade inflammation is a hallmark of diabetes and obesity. Therefore, the anti-inflammatory effects of sPIF could be beneficial in these diseases, particularly its potential prophylactic or therapeutic effects on insulin secretion. However, in this study, sPIF was able to improve insulin secretion in human islets and Min-6 cells lacking an inflammatory milieu. These data lead us to propose that, at least in part, the mechanism of action of sPIF on insulin secretion is beyond its proposed anti-inflammatory effects. On the other hand, prophylactic use of sPIF has raised expectations in the setting of T1D.[9] Thus, 16 weeks of sPIF treatment in NOD mice delayed the onset of diabetes for up to 14 weeks postadministration, preserving islet cytoarchitecture and insulin staining, which is associated with a reduction in oxidative stress and β-cell apoptosis.[9]

Of note, sPIF has been involved in the regulation of pancreatic islets architecture. Weiss et al. showed that continuous administration of sPIF prevented the onset of diabetes in an acute adoptive-transfer model and in the NOD mouse model.[9] The effects of sPIF were associated with reduced actin and tubulin proteins, thereby blocking neutrophil invasion and inflammation. Interestingly, PIF also targets actin and tubulin cytoskeletal proteins in cells of the immune system.[60] We have recently described that the specific deletion of IDE in pancreatic α-cells resulted in constitutive glucagon secretion, as well as α-cells hypertrophy and hyperplasia.[33] The dysregulation of glucagon secretion seen in α-cells was associated with decreased levels of tubulin and acetylated tubulin leading to cytoskeleton disorganization, indicating that IDE is required for tubulin cytoskeleton dynamics and vesicular trafficking. This mechanism of action may also be compatible with the constitutive insulin secretion observed in deficient-IDE β-cells from the B-IDE-KO mouse.[32] In the present work, we have showed that sPIF increases insulin secretion in β-cells. Two possible hypotheses may explain the mechanism(s) by which sPIF increases insulin secretion in β-cells. First, similar to what happens in α-cells, IDE may mediate remodelling of cytoskeletal architecture through regulation of microtubules dynamics, which would result in increased GSIS in β-cells. Second, we have observed an increase in the gene expression of the K_{ATP} subunits in β-cells after sPIF treatment, suggesting that sPIF could upregulate K_{ATP}-channel expression. K_{ATP} channel activity is a key regulator of glucose-induced insulin secretion; this mechanism is finely balanced. Both increased and decreased K_{ATP} channel activity have been reported to lead to a reduction in insulin secretion.[61] Modifications in K_{ATP} subunits expression does not necessarily translate into modulation of channel activity. However, interaction between K_{ATP} subunits and the actin cytoskeleton has been shown in other models.[62] Therefore, we cannot rule out the possibility that a synergy of both mechanisms, cytoskeleton dynamics and K_{ATP} channel modulation, would lead to an improvement of insulin

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secretion in response to sPIF in β-cells. Nevertheless, further studies are needed to corroborate these mechanisms of action of sPIF on insulin secretion.

Glucose-lowering therapy significantly contributes to diabetes management. Although successful therapies such as metformin, glucagon-like peptide-1 receptor agonists and sodium-glucose co-transporter-2 inhibitors can provide improved glycaemic control, it is necessary to develop novel treatment options with fewer and less severe adverse drug effects (e.g. weight gain and hypoglycaemia). Furthermore, it is only recently that the characteristics and presence of co-morbidities in individual patients have been taken into consideration. Multidrug therapy is often recommended when the first-line therapy proves to be ineffective. Because sPIF is clinically safe and well tolerated, we hypothesize that its administration in combination with current treatments will help to develop personalized approaches and expand the therapeutic options for glucose-lowering drugs in different subgroups of patients. Additionally, further research is warranted to show that sPIF can overcome some of the limitations of the current therapies, such as short half-lifes, frequent dosing and low bioavailability.

In conclusion, our results show that sPIF specifically targets proteolytic activation of IDE in pancreatic β-cells, resulting in an increased capacity for insulin secretion. In a preclinical mouse model of obesity and T2D, this effect of sPIF on pancreatic islets results in improved glucose tolerance. Therefore, sPIF is a promising secretagogue for the treatment of T2D.

AUTHOR CONTRIBUTIONS

AS, IC, GP and BM made substantial contributions to conception and design, acquisition or analysis and interpretation of data. GA synthesized the peptide. IC, GP and BM prepared the original draft of the manuscript and AS, IC, CB, JS, MR, CH, TT, GP and BM critically revised it for important intellectual content. All authors accepted the final version to be published. GP and BM are responsible for the integrity of the work as a whole.

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CONFLICT OF INTEREST

The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data available on request from the authors

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REFERENCES

1. Coulam CB, Roussev RG, Thomason EJ, Barnea ER. Preimplantation factor (PIF) predicts subsequent pregnancy loss. *Am J Reprod Immunol*. 1995;34(2):88-92. doi:10.1111/j.1600-0897.1995.tb00923.x
2. Barnea ER. Applying embryo-derived immune tolerance to the treatment of immune disorders. *Ann N Y Acad Sci*. 2007;1110:602-618. doi:10.1196/annals.1423.064
3. Barnea ER, Kirk D, Paidas MJ. Preimplantation factor (PIF) promoting role in embryo implantation: increases endometrial integrin-alpha2-beta3, amphiregulin and epiregulin while reducing betacellulin expression via MAPK in decidua. *Reprod Biol Endocrinol*. 2012;10:50. doi:10.1186/1477-7827-10-50
4. Stamatkin CW, Roussev RG, Stout M, et al. PreImplantation factor (PIF) correlates with early mammalian embryo development-bovine and murine models. *Reprod Biol Endocrinol*. 2011;9:63. doi:10.1186/1477-7827-9-63
5. Ramu S, Stamatkin C, Timms L, Ruble M, Roussev RG, Barnea ER. PreImplantation factor (PIF) detection in maternal circulation in early pregnancy correlates with live birth (bovine model). *Reprod Biol Endocrinol*. 2013;11:105. doi:10.1186/1477-7827-11-105
6. Duzyj CM, Barnea ER, Li M, Huang SJ, Krikun G, Paidas MJ. Preimplantation factor promotes first trimester trophoblast invasion. *Am J Obstet Gynecol*. 2010;203(4):402. doi:10.1016/j.ajog.2010.06.060
7. Zare F, Seifati SM, Dehghan-Manshadi M, Fesahat F. Preimplantation factor (PIF): a peptide with various functions. *JBRA Assist Reprod*. 2020;24(2):214-218. doi:10.5935/1518-0557.20190082
8. O'Brien CB, Barnea ER, Martin P, et al. Randomized, double-blind, placebo-controlled, single ascending dose trial of synthetic preimplantation factor in autoimmune hepatitis. *Hepatol Commun*. 2018;2(10):1235-1246. doi:10.1002/hep4.1239
9. Weiss L, Bernstein S, Jones R, et al. Preimplantation factor (PIF) analog prevents type I diabetes mellitus (TIDM) development by preserving pancreatic function in NOD mice. *Endocrine*. 2011;40(1):41-54. doi:10.1007/s12020-011-9438-5

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10. Chen YC, Rivera J, Fitzgerald M, et al. PreImplantation factor prevents atherosclerosis via its immunomodulatory effects without affecting serum lipids. *Thromb Haemost*. 2016;115(5):1010-1024. doi:10.1160/TH15-08-0640
11. Leissring MA, Gonzalez-Casimiro CM, Merino B, Suire CN, Perdomo G. Targeting insulin-degrading enzyme in insulin clearance. *Int J Mol Sci*. 2021;22(5):2235. doi:10.3390/ijms22052235
12. Gonzalez-Casimiro CM, Merino B, Casanueva-Alvarez E, et al. Modulation of insulin sensitivity by insulin-degrading enzyme. *Biomedicine*. 2021;9(1):86. doi:10.3390/biomedicines9010086
13. Paidas MJ, Krikun G, Huang SJ, et al. A genomic and proteomic investigation of the impact of preimplantation factor on human decidual cells. *Am J Obstet Gynecol*. 2010;202(5):459. doi:10.1016/j.ajog.2010.03.024
14. Hayrabedyan S, Todorova K, Spinelli M, Barnea ER, Mueller M. The core sequence of PIF competes for insulin/amyloid beta in insulin degrading enzyme: potential treatment for Alzheimer's disease. *Oncotarget*. 2018;9(74):33884-33895. doi:10.18632/oncotarget.26057
15. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ. A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes*. 1999;48(5):1175-1182. doi:10.2337/diabetes.48.5.1175
16. Groves CJ, Wiltshire S, Smedley D, et al. Association and haplotype analysis of the insulin-degrading enzyme (IDE) gene, a strong positional and biological candidate for type 2 diabetes susceptibility. *Diabetes*. 2003;52(5):1300-1305. doi:10.2337/diabetes.52.5.1300
17. Karamohamed S, Demissie S, Volcjak J, et al. Polymorphisms in the insulin-degrading enzyme gene are associated with type 2 diabetes in men from the NHLBI Framingham Heart Study. *Diabetes*. 2003;52(6):1562-1567. doi:10.2337/diabetes.52.6.1562
18. Gu HF, Efendic S, Nordman S, et al. Quantitative trait loci near the insulin-degrading enzyme (IDE) gene contribute to variation in plasma insulin levels. *Diabetes*. 2004;53(8):2137-2142. doi:10.2337/diabetes.53.8.2137
19. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature*. 2007;445(7130):881-885. doi:10.1038/nature05616
20. Kullenberg H, Rossen J, Johansson UB, et al. Increased levels of insulin-degrading enzyme in patients with type 2 diabetes mellitus. *Endocrine*. 2022;77(3):561-565. doi:10.1007/s12020-022-03123-7
21. Sofer Y, Nash Y, Osher E, et al. Insulin-degrading enzyme higher in subjects with metabolic syndrome. *Endocrine*. 2021;71(2):357-364. doi:10.1007/s12020-020-02548-2
22. Fosam A, Sikder S, Abel BS, et al. Reduced insulin clearance and insulin-degrading enzyme activity contribute to hyperinsulinemia in African Americans. *J Clin Endocrinol Metab*. 2020;105(4):e1835-e1846. doi:10.1210/clinem/dgaa070
23. Pivovarova O, von Loeffelholz C, Ilkavets I, et al. Modulation of insulin degrading enzyme activity and liver cell proliferation. *Cell Cycle*. 2015;14(14):2293-2300. doi:10.1080/15384101.2015.1046647
24. Fernandez-Diaz CM, Escobar-Curbelo L, Lopez-Acosta JF, et al. Insulin degrading enzyme is up-regulated in pancreatic beta cells by insulin treatment. *Histol Histopathol*. 2018;33(11):1167-1180. doi:10.14670/HH-11-997
25. Merino B, Fernandez-Diaz CM, Parrado-Fernandez C, et al. Hepatic insulin-degrading enzyme regulates glucose and insulin homeostasis in diet-induced obese mice. *Metabolism*. 2020;113:154352. doi:10.1016/j.metabol.2020.154352
26. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412-419. doi:10.1007/BF00280883
27. Fraulob JC, Ogg-Diamantino R, Fernandes-Santos C, Aguila MB, Mandarim-de-Lacerda CA. A mouse model of metabolic syndrome: insulin resistance, fatty liver and non-alcoholic fatty pancreas disease (NAFPD) in C57BL/6 mice fed a high fat diet. *J Clin Biochem Nutr*. 2010;46(3):212-223. doi:10.3164/jcbn.09-83
28. Rong X, Li Y, Ebihara K, et al. Angiotensin II type 1 receptor-independent beneficial effects of telmisartan on dietary-induced obesity, insulin resistance and fatty liver in mice. *Diabetologia*. 2010;53(8):1727-1731. doi:10.1007/s00125-010-1744-6
29. Xu W, Li J, Ji C, et al. Activation of POMC neurons to adiponectin participating in EA-mediated improvement of high-fat diet IR mice. *Front Neurosci*. 2023;21(17):1145079. doi:10.3389/fnins.2023.1145079
30. Kurauti MA, Ferreira SM, Soares GM, et al. Hyperinsulinemia is associated with increasing insulin secretion but not with decreasing insulin clearance in an age-related metabolic dysfunction mice model. *J Cell Physiol*. 2019;234(6):9802-9809. doi:10.1002/jcp.27667
31. Kurauti MA, Costa-Júnior JM, Ferreira SM, et al. Acute exercise restores insulin clearance in diet-induced obese mice. *J Endocrinol*. 2016;229(3):221-232. doi:10.1530/JOE-15-0483
32. Fernández-Díaz CM, Merino B, López-Acosta JF, et al. Pancreatic β-cell-specific deletion of insulin-degrading enzyme leads to dysregulated insulin secretion and β-cell functional immaturity. *Am J Physiol Endocrinol Metab*. 2019;317(5):E805-E819. doi:10.1152/ajpendo.00040.2019
33. Merino B, Casanueva-Álvarez E, Quesada I, et al. Insulin-degrading enzyme ablation in mouse pancreatic alpha cells triggers cell proliferation, hyperplasia and glucagon secretion dysregulation. *Diabetologia*. 2022;65(8):1375-1389. doi:10.1007/s00125-022-05729-y
34. González-Casimiro CM, Cámara-Torres P, Merino B, et al. Effects of fasting and feeding on transcriptional and posttranscriptional regulation of insulin-degrading enzyme in mice. *Cell*. 2021;10(9):2446. doi:10.3390/cells10092446
35. Maianti JP, McFedries A, Foda ZH, et al. Anti-diabetic activity of insulin-degrading enzyme inhibitors mediated by multiple hormones. *Nature*. 2014;511(7507):94-98. doi:10.1038/nature13297
36. Abdul-Hay SO, Kang D, McBride M, Li L, Zhao J, Leissring MA. Deletion of insulin-degrading enzyme elicits antipodal, age-dependent effects on glucose and insulin tolerance. *PloS One*. 2011;6(6):e20818. doi:10.1371/journal.pone.0020818
37. Pascoe L, Tura A, Patel SK, et al. RISC Consortium; U.K. Type 2 Diabetes Genetics ConsortiumCommon variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. *Diabetes*. 2007;56(12):3101-3104. doi:10.2337/db07-0634
38. Grarup N, Rose CS, Andersson EA, et al. Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. *Diabetes*. 2007;56(12):3105-3111. doi:10.2337/db07-0856
39. Steneberg P, Bernardo L, Edfalk S, et al. The type 2 diabetes-associated gene ide is required for insulin secretion and suppression of α-synuclein levels in β-cells. *Diabetes*. 2013;62(6):2004-2014. doi:10.2337/db12-1045
40. Fakhrai-Rad H, Nikoshkov A, Kamel A, et al. Insulin-degrading enzyme identified as a candidate diabetes susceptibility gene in GK rats. *Hum Mol Genet*. 2000;9(14):2149-2158. doi:10.1093/hmg/9.14.2149
41. Kraupner N, Dinh CP, Wen X, et al. Identification of indole-based activators of insulin degrading enzyme. *Eur J Med Chem*. 2022;15(228):113982. doi:10.1016/j.ejmech.2021.113982
42. Durham TB, Toth JL, Klimkowski VJ, et al. Dual exosite-binding inhibitors of insulin-degrading enzyme challenge its role as the primary mediator of insulin clearance in vivo. *J Biol Chem*. 2015;290(33):20044-20059. doi:10.1074/jbc.M115.638205
43. Nash Y, Ganoth A, Borenstein-Auerbach N, et al. From virus to diabetes therapy: characterization of a specific insulin-degrading enzyme

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inhibitor for diabetes treatment. *FASEB J*. 2021;35(5):e21374. doi:10.1096/fj.201901945R
44. Selkoe DJ. Clearing the brain's amyloid cobwebs. *Neuron*. 2001;32(2):177-180. doi:10.1016/s0896-6273(01)00475-5
45. Adessi C, Enderle T, Grueninger F, Roth D. Activator for Insulin Degrading Enzyme. 2006 WO2006066847.
46. Cabrol C, Huzarska MA, Dinolfo C, et al. Small-molecule activators of insulin-degrading enzyme discovered through high-throughput compound screening. *PLoS One*. 2009;4(4):e5274. doi:10.1371/journal.pone.0005274
47. Kukday SS, Manandhar SP, Ludley MC, Burriss ME, Alper BJ, Schmidt WK. Cell-permeable, small-molecule activators of the insulin-degrading enzyme. *J Biomol Screen*. 2012;17(10):1348-1361. doi:10.1177/1087057112451921
48. Çakir B, Dağliyan O, Dağyildiz E, et al. Structure based discovery of small molecules to regulate the activity of human insulin degrading enzyme. *PLoS One*. 2012;7(2):e31787. doi:10.1371/journal.pone.0031787
49. Villa-Pérez P, Merino B, Fernández-Díaz CM, et al. Liver-specific ablation of insulin-degrading enzyme causes hepatic insulin resistance and glucose intolerance, without affecting insulin clearance in mice. *Metabolism*. 2018;88:1-11. doi:10.1016/j.metabol.2018.08.001
50. Borges DO, Patarrão RS, Ribeiro RT, et al. Loss of postprandial insulin clearance control by insulin-degrading enzyme drives dysmetabolism traits. *Metabolism*. 2021;118:154735. doi:10.1016/j.metabol.2021.154735
51. Piccinini F, Bergman RN. The measurement of insulin clearance. *Diabetes Care*. 2020;43(9):2296-2302. doi:10.2337/dc20-0750
52. Polonsky KS, Rubenstein AH. C-peptide as a measure of the secretion and hepatic extraction of insulin. Pitfalls and limitations. *Diabetes*. 1984;33(5):486-494. doi:10.2337/diab.33.5.486
53. Ehrhardt N, Cui J, Dagdeviren S, et al. Adiposity-independent effects of aging on insulin sensitivity and clearance in mice and humans. *Obesity (Silver Spring)*. 2019;27(3):434-443. doi:10.1002/oby.22418
54. Wonfor RE, Creevey CJ, Natoli M, Hegarty M, Nash DM, Rose MT. Interaction of preimplantation factor with the global bovine endometrial transcriptome. *PloS One*. 2020;15(12):e0242874. doi:10.1371/journal.pone.0242874
55. Ambrożkiewicz KA, Kozłowska U, Haesler V, Barnea ER, Mueller M, Kurpisz M. Murine glial progenitor cells transplantation and synthetic PreImplantation factor (sPIF) reduces inflammation and early motor impairment in ALS mice. *Sci Rep*. 2022;12(1):4016. doi:10.1038/s41598-022-08064-9
56. Hayrabedyan S, Shainer R, Yekhtin Z, et al. Synthetic PreImplantation factor (sPIF) induces posttranslational protein modification and reverses paralysis in EAE mice. *Sci Rep*. 2019;9(1):12876. doi:10.1038/s41598-019-48473-x
57. Mueller M, Schoeberlein A, Zhou J, et al. PreImplantation factor bolsters neuroprotection via modulating protein kinase a and protein kinase C signaling. *Cell Death Differ*. 2015;22(12):2078-2086. doi:10.1038/cdd.2015.55
58. Spinelli M, Boucard C, Di Nicuolo F, et al. Synthetic PreImplantation factor (sPIF) reduces inflammation and prevents preterm birth. *PloS One*. 2020;15(6):e0232493. doi:10.1371/journal.pone.0232493
59. Roussev RG, Dons'koi BV, Stamatkin C, et al. Preimplantation factor inhibits circulating natural killer cell cytotoxicity and reduces CD69 expression: implications for recurrent pregnancy loss therapy. *Reprod Biomed Online*. 2013;26(1):79-87. doi:10.1016/j.rbmo.2012.09.017
60. Barnea ER, Hayrabedyan S, Todorova K, et al. PreImplantation factor (PIF*) regulates systemic immunity and targets protective regulatory and cytoskeleton proteins. *Immunobiology*. 2016;221(7):778-793. doi:10.1016/j.imbio.2016.02.004
61. Ashcroft FM. ATP-sensitive potassium channelopathies: focus on insulin secretion. *J Clin Invest*. 2005;115(8):2047-2058. doi:10.1172/JCI25495
62. Yokoshiki H, Katsube Y, Sunugawa M, Seki T, Sperelakis N. Disruption of Actin cytoskeleton attenuates sulfonylurea inhibition of cardiac ATP-sensitive K+ channels. *Pflugers Arch*. 1997;434(2):203-205. doi:10.1007/s004240050384

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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