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Nicotinamide phosphoribosyltransferase enhances beta cell expansion during pregnancy

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Abstract. – OBJECTIVE: Many factors contribute to the development of gestational diabetes mellitus (GDM). Among these factors, nicotinamide phosphoribosyltransferase (NAMPT) plays a critical role. Previous studies have demonstrated the effects of NAMPT on insulin resistance during GDM, while the effects of NAMPT on beta cells remain unknown. Here we addressed this question.

PATIENTS AND METHODS: Mouse islets were isolated and transduced with adeno-associated vectors carrying NAMPT. Glucose-stimulated insulin secretion, beta cell proliferation, and beta cell apoptosis were analyzed in NAMPT-overexpressing islets, compared to controls.

RESULTS: Overexpression of NAMPT in primary mouse islets increased glucose-stimulated insulin secretion and increased beta cell expansion through augmentation of beta cell proliferation, without affecting beta cell apoptosis.

CONCLUSIONS: NAMPT enhances expansion of beta cell mass during pregnancy. Inadequate NAMPT may contribute to the development of GDM partially through reduced beta cell expansion in the gestational period.

Key Words:
Nicotinamide phosphoribosyltransferase, Gestational diabetes mellitus, Beta cell proliferation, Beta cell function.

Abbreviation

GDM = gestational diabetes mellitus; NAMPT = Nicotinamide phosphoribosyltransferase; ELISA = enzyme-linked immunosorbent assay

Introduction

Diabetes mellitus is characterized by chronic hyperglycaemia, which results from impairment of glucose metabolisms by dysfunction or insufficiency of insulin-secreting beta cells in the pancreas[1-4]. An adequate and properly functional beta cell mass is necessary for maintaining glucose-stimulated insulin release in response to the metabolic need. During pregnancy, the requirement for glucose metabolism significantly increases, which requires increases in beta-cell mass[5]. Most previous studies have shown that postnatal increases in beta cell mass, including gestation[6-13], are through beta cell replication[11,14-18]. Therefore, the defect in gestational beta cell proliferation, as well as defect in insulin secretion, may induce dysfunction of glucose metabolism, and even gestational diabetes mellitus (GDM).

GDM is a very common complication during gestation, and nearly 10% of pregnancies worldwide was affected by GDM[19-23], the severe cases from which have been found to lead to maternal death and perinatal morbidity. Different factors regulate the development of GDM. Among all these factors, nicotinamide phosphoribosyltransferase (NAMPT) has recently been recognized as an important one. Fukuhara et al[24] compared paired samples of subcutaneous fat and visceral fat, and they found that plasma NAMPT concentrations strongly correlated with the amount of visceral fat whereas only weakly with the amount of subcutaneous fat. Using heterozygous NAMPT gene knockout mice, they provide evidence that like insulin, NAMPT binds to insulin receptor in a non-competitive way with insulin to lower down plasma glucose levels, possibly through enhancement of glucose uptake in adipocytes and suppression of glucose release in hepatocytes[24]. However, the effects of NAMPT on beta cells remain unknown.

Here, we specifically focused the effects of NAMPT on beta cells. Mouse islets were isolated and overexpressed with NAMPT. Glucose-stimulated insulin secretion, beta cell proliferation, and beta cell apoptosis were analyzed in NAMPT-overexpressing islets, compared to control islets.

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Our findings suggest that NAMPT may enhance expansion of beta cell mass during pregnancy. Inadequate NAMPT may contribute to the development of GDM partially through reduced beta cell expansion in the gestational period.

Materials and Methods

Experimental Protocol Approval

The experiments were performed in accordance with the ethical principles for medical research outlined in the Declaration of Helsinki 1964. All experimental protocols were approved by the Research Bureau of Hebei People's Hospital. The methods regarding human specimens were carried out in "accordance" with the approved institutional guidelines from Hebei People's Hospital by the Ethics Committee. All mouse experiments were approved and conducted in accordance with the Institutional Animal Care and Use Committee at Hebei People's Hospital.

Mouse Islets

Female Balb/C mice were purchased from Jackson Labs (Bar Harbor, ME, USA) and the islets from these mice were harvested at 12 weeks of age, as described[25,26]. The purity of the mouse islets was confirmed by examination of insulin and amylase transcripts, compared to the total pancreas. The islet perifusion was performed as described before[26].

Infection of Mouse Islets

Two adeno-associated viruses (AAVs) were prepared to carry NAMPT and a GFP reporter or NAMPT only for transduction of primary mouse islets. The preparation of AAVs has been described previously[27-29].

Beta Cell Proliferation and Apoptosis

For analysis and quantification of beta-cell proliferation, Ki-67 and insulin double immunostaining were performed on primary mouse islets in culture, as has been described [30]. For analysis and quantification of beta-cell apoptosis, TUNEL staining was performed, using In Situ Cell Death Detection Kit (Roche, Nutley, NJ, USA).

Western Blot

Western blot was performed as described[31]. The primary antibodies are rabbit anti-NAMPT and anti-GAPDH (Cell Signaling, San Jose, CA, USA). GAPDH was used as a protein loading control. Secondary antibody is HRP-conjugated anti-rabbit (Jackson Labs, Bar Harbor, ME, USA). The representative image was shown. Densitometry of Western blots was quantified with NIH ImageJ software (Bethesda, MA, USA).

Quantitative Real-time PCR (RT-qPCR)

RNA was extracted with Trizol (Invitrogen, Carlsbad, CA, USA) for cDNA synthesis. Quantitative real-time PCR were performed in duplicates with QuantiTect SYBR Green PCR Kit (Qiagen). NAMPT primers are: forward: 5'-ATGAATCCTGCGGCAGAAGC -3' and reverse: 5'-CTAATGATGTGCTGCTTCCAGT-3'. GAPDH primers are: forward: 5'- GTGTTCCTACCCCCAATGTGT-3' and reverse: 5'-ATTGTCATACCAGGAAATGAGCTT-3'. Values of NAMPT were first normalized against GAPDH, and then compared to the control condition.

Statistical Analysis

All values are depicted as mean ± standard deviation from at least 5 individuals and are considered significant if $p < 0.05$. All data were statistically analyzed using one-way ANOVA with a Bonferroni correction.

Results

Moues Islet Isolation

Here, we aimed to examine the role of NAMPT on beta cells. Thus, we isolated mouse islets (Figure 1A) and checked the purity of the islet fraction by insulin (a marker for beta cells) and amylase (a marker for exocrine acinar cells that compose more than 85% of all pancreatic cells). We found that the insulin transcripts in the islet fraction was enriched by 25 times (Figure 1B), while the amylase transcripts in the islet fraction was reduced by 95% (Figure 1C), suggesting that the islet fraction is quite pure and lack of contamination of exocrine pancreatic cells.

Transduction of Mouse Islets

Mouse islets were transduced with AAV-NAMPT-GFP (simplified as NAMPT) or AAV-GFP (simplified as CTL). The overexpression of NAMPT by AAV-NAMPT-GFP was confirmed by RT-qPCR (Figure 2A) and by Western blot (Figure 2B).

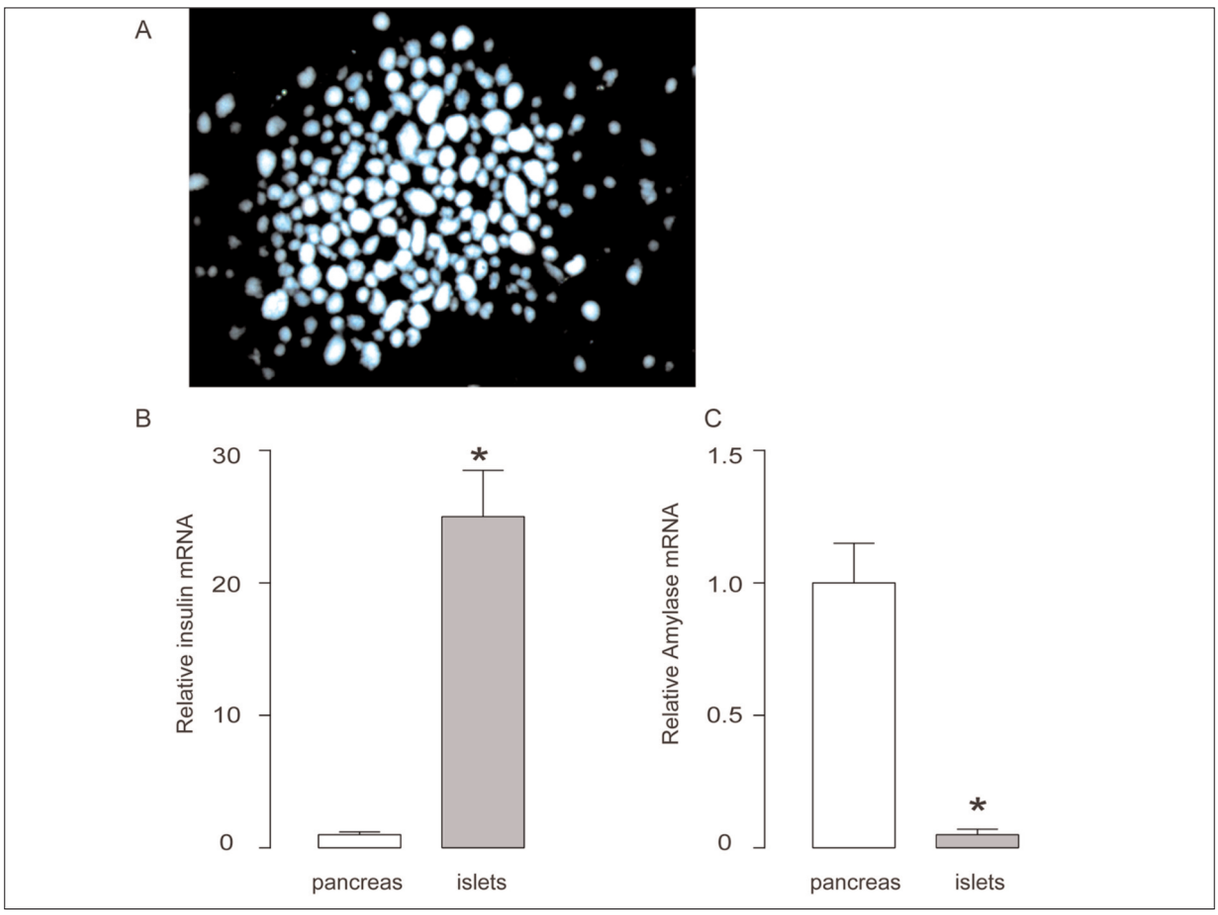

Figure 1. Moues Islet isolation. ***A,*** Isolated mouse islets in culture. ***B,-C,*** RT-qPCR for insulin ***(B)*** and amylase ***(C)*** in total pancreas or isolated islets. N = 5. *$p < 0.05$.

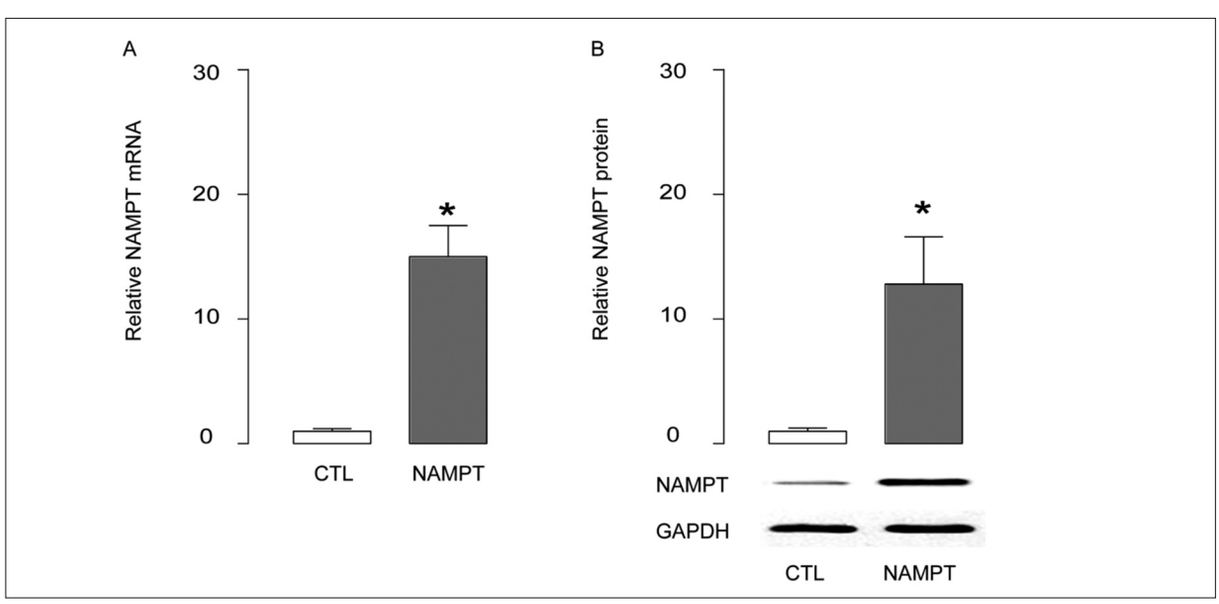

Figure 2. Transduction of mouse islets. ***A,-B,*** Mouse islets were transduced with AAV-NAMPT-GFP (simplified as NAMPT) or AAV-GFP (simplified as CTL). NAMPT levels were determined by RT-qPCR ***(A)***, and by Western blot ***(B)***. N = 5. *$p < 0.05$.

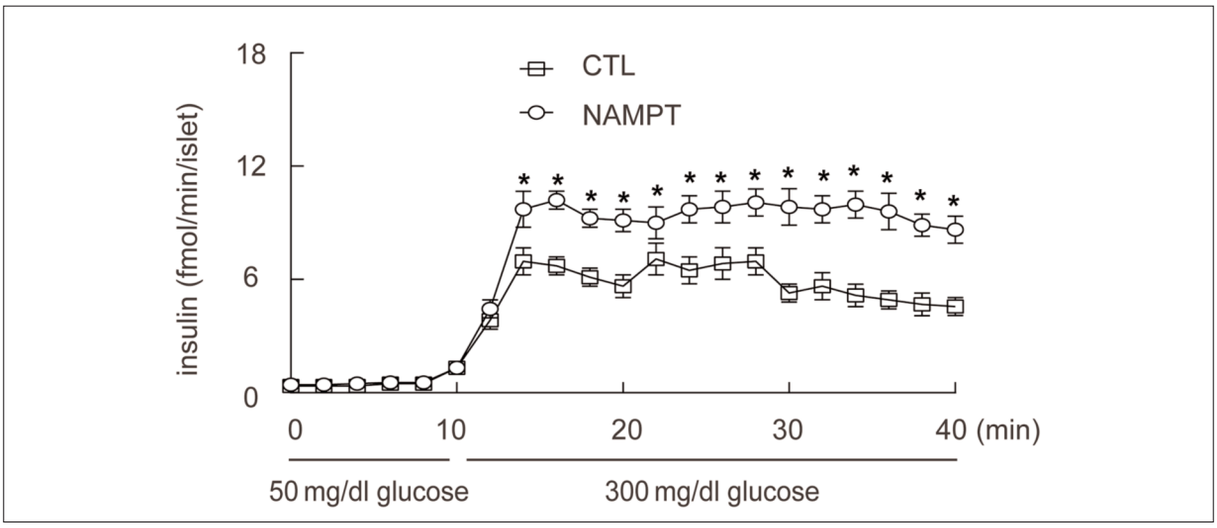

Figure 3. NAMPT enhances insulin secretion of mouse islets. Islet perifusion was performed using mouse islets that overexpress NAMPT (NAMPT), compared with control islets (CTL). N = 5. *$p < 0.05$.

NAMPT Enhances Insulin Secretion of Mouse Islets

We found that overexpression of NAMPT in mouse islets increased the insulin secretion (Figure 3), suggesting that NAMPT may improve islet function.

NAMPT Increases Beta Cell Proliferation Without Affecting Apoptosis

We then analyzed the effects of NAMPT on beta cell proliferation and apoptosis by Ki-67 assay and TUNEL assay, respectively. We found that overexpression of NAMPT in mouse islets significantly increased proliferating beta cells, shown by representative images (Figure 4A), and by quantification (Figure 4B). Moreover, overexpression of NAMPT in mouse islets did not alter cell apoptosis (Figure 4C). These data suggest that NAMPT may be critical during gestation. NAMPT promotes both beta cell proliferation and insulin secretion, which are important for the body to respond properly to the increased metabolic need (Figure 5).

Hence, low NAMPT levels may predispose the development of GDM, at least partially through impairment of beta cell expansion during gestation as well as insulin secretion by beta cells.

Discussion

NAMPT is an adipocytokine of pleiotropic effects, and NAMPT plays a critical role in body metabolism and immunity. Originally identified as a pre-B-cell colony-enhancing factor produced by lymphocytes, NAMPT was later found to be expressed in almost every tissue of the human body, among which visceral fat appeared to be the main source.

Many reports have been published for circulating NAMPT levels in different pathophysiological metabolic conditions. Moreover, the maternal plasma concentration of NAMPT increases during pregnancy. However, the exact role of NAMPT in metabolic changes is not decided yet. In the original paper, Fukuhara et al[24] reported a correlation between amounts of visceral fat and levels of plasma NAMPT, which was supported by some follow-up studies confirming a potential connection between plasma NAMPT levels and anthropometric and metabolic parameters in type 1 diabetes, type 2 diabetes, and GDM[32-35]. However, the effects of NAMPT on beta cells remain unknown.

The level of insulin resistance is also an important factor for the development of GDM, and NAMPT has been extensively studied for their effects on peripheral tissue insulin responses in the previous studies. Thus, we just focused on the effects of NAPMT on beta cells here.

Conclusions

We studied the NAMPT in GDM patients and then we specifically analyzed the effects of NAMPT on beta cells. Adequate functional beta-

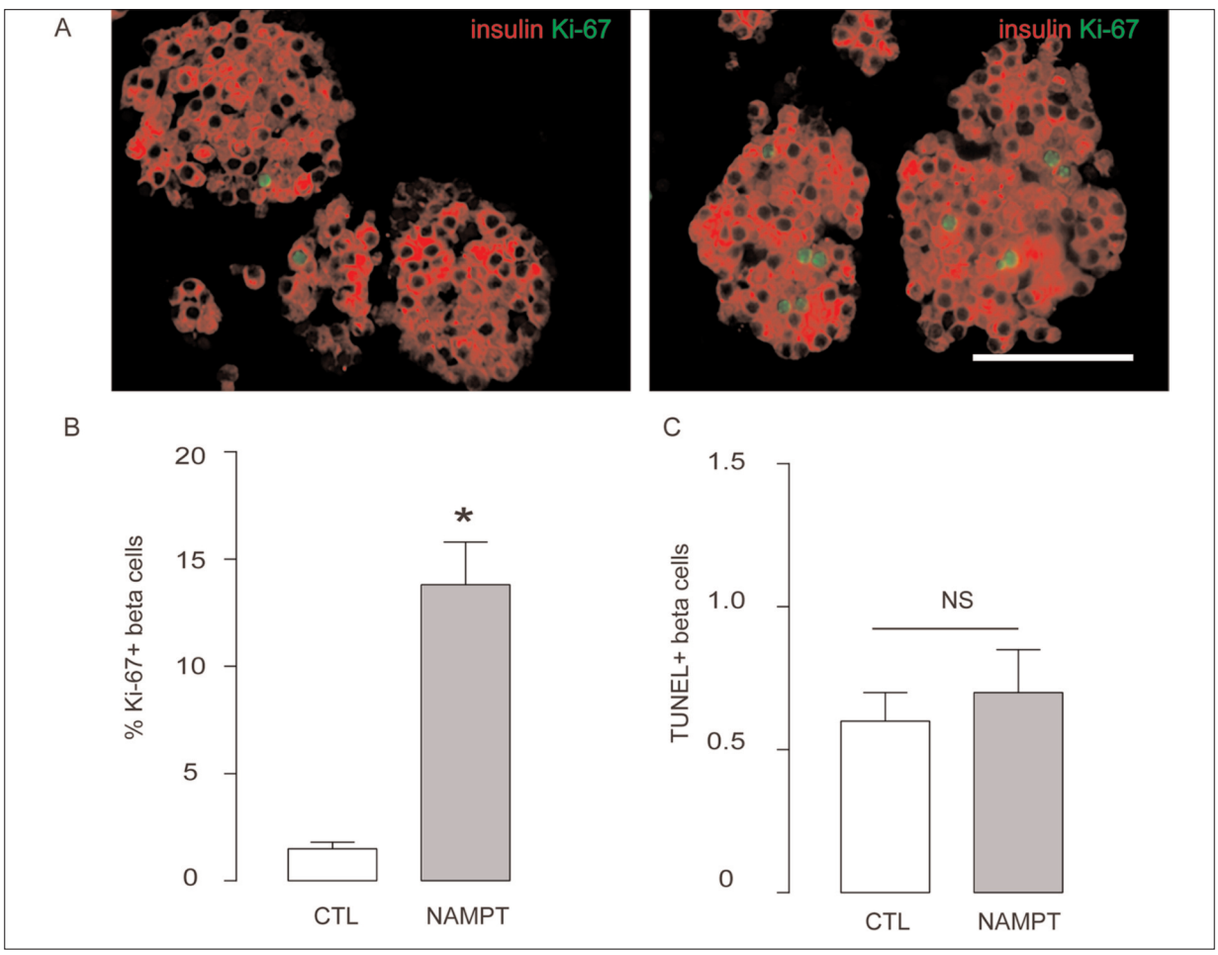

Figure 4. NAMPT increases beta cell proliferation without affecting apoptosis. ***A,-B,*** Beta cell proliferation by Ki-67 and insulin staining, shown by representative images ***(A)***, and by quantification ***(B)***. ***C,*** Quantification of beta cell apoptosis by TUNEL assay. N = 5. *$p < 0.05$. NS: non-significant. Scale bar is 50 μm.

cell mass is important for maintaining proper control of blood sugar in the body. Since beta-cell mass increase dramatically during gestation and many evidence have shown that failure of augmentation of beta cell mass leads to GDM[8]. The serum NAMPT levels were significantly lower in GDM patients than in pregnant women without GDM. *In vitro*, we found that in purified mouse islets, NAMPT not only increased insulin secretion, but also induced beta cell proliferation.

Thus, NAMPT is critical for an adequate response from beta cells to the increased metabolic need during gestation. These data indicate that NAMPT may enhance expansion of beta cell mass during pregnancy. Inadequate NAMPT may contribute to the development of GDM partially through reduced beta cell expansion in the gestational period.

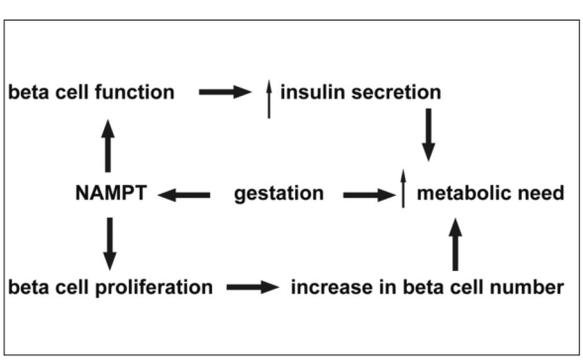

Figure 5. Schematic of the model. NAMPT promotes both beta cell proliferation and insulin secretion, which are important for the body to respond properly to the increased metabolic need. Low NAMPT levels may predispose development of GDM, at least partially through impairment of beta cell expansion during gestation as well as insulin secretion by beta cells.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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