Suppression of Hepatic Glucose Production by Human Neutrophil α-Defensins through a Signaling Pathway Distinct from Insulin*

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In this study, we tested the hypothesis that human neutrophil α-defensins (HNPs) inhibit hepatic glucose production through a signaling pathway distinct from insulin. The effect of HNP-1 on fasting glucose levels and the expression of hepatic gluconeogenic genes was first examined. Using hyperinsulineemic-euglycemic clamps, we determined the effect of HNP-1 on endogenous glucose production, hepatic expression of key gluconeogenic genes and glucose uptake in skeletal muscle in Zucker diabetic fatty rats. In isolated primary hepatocytes, we studied the effect of HNP-1 and -2 on glucose production, expression of gluconeogenic genes, and phosphorylation of Akt, c-Src, and FoxO1. Our results show that HNP-1 reduced blood glucose levels of both normal mice and Zucker diabetic fatty rats predominantly through suppression of hepatic glucose production. HNPs inhibited glycogenolysis and gluconeogenesis in isolated hepatocytes. HNPs also suppressed expression of key gluconeogenic genes including phosphoenoylpyruvate carboxyl kinase and glucose-6-phosphatase. To investigate the mechanism, we found that HNPs stimulated phosphorylation of Akt and FoxO1 without activating IRS1. Nevertheless, HNPs activated c-Src. Blockade of c-Src activity with either a chemical inhibitor PP2 or an alternative inhibitor CSK prevented the inhibitory effect of HNPs on gluconeogenesis. Together, our results support the hypothesis that HNPs can suppress hepatic glucose production through an intracellular mechanism distinct from the classical insulin signaling pathway.

Hepatic glucose production, including gluconeogenesis and glycogenolysis, is essential for maintaining blood glucose levels during fasting and is a major contributor to hyperglycemia in diabetes, which afflicts a large portion of the American population and continues to increase in its incidence and negative impact on the general health and overall economy worldwide (1, 2). Normally, blood glucose levels fluctuate within a very narrow range because of tight control of hepatic gluconeogenesis by various hormones (3, 4). Some hormones including glucagon and glucocorticoids stimulate, whereas others, including insulin and adiponectin, inhibit hepatic gluconeogenesis (1, 5). Although increases in circulating levels of the stimulatory hormones and decreases in some inhibitory hormones may contribute to the unrestrained hepatic gluconeogenesis and hyperglycemia in diabetes, the primary cause of diabetes is the loss of insulin function because of deficient insulin production (type I) or defective insulin action (type II) (1, 6, 7). Therefore, the standard treatment of diabetes is either to restore insulin function by providing insulin or to improve insulin action with insulin sensitizers such as thiazolidinediones or other agents that can activate Akt, a necessary component of insulin signaling, but bypass the blunted upstream components such as IRS1/2 of the classical insulin signaling pathway.

In searching for agents like insulin that can activate Akt and consequently suppress hepatic gluconeogenesis, we considered human neutrophil peptides (HNP-1, HNP-2, HNP-3, and HNP-4), which belong to the α subfamily of defensins (8, 9). In addition to their primary function in innate immunity, defensins have recently been shown to be involved in other fundamental cellular activities such as adhesion and proliferation of vascular endothelial cells (10, 11), wound healing (12), and angiogenesis (10, 13). In our previous work in lung epithelial cells and monocytes, we have observed that HNPs can activate Akt (14), which is a necessary component for insulin signaling (reviewed in Refs. 1 and 15). Furthermore, plasma levels of HNPs can increase more than a thousand times in patients with severe bacterial infections, and interestingly these instances are frequently accompanied by severe hypoglycemia and suppressed hepatic gluconeogenesis (16–20). However, it is unknown whether the increase of plasma HNPs is involved in the suppression of hepatic gluconeogenesis by bacterial infections. Others have shown that plasma levels of certain defensins

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‡‡ The abbreviations used are: HNP, human neutrophil peptide; GFP, green fluorescent protein; PEPCK, phosphoenoylpyruvate carboxyl kinase; G6Pase, glucose-6-phosphatase; ZDF, Zucker diabetic fatty; LDH, lactate dehydrogenase; EGP, endogenous glucose production; 2-[14C]DG, 2-deoxy-D-[1-14C]glucose; PGC-1α, peroxisome proliferator-activated receptor coactivator-1α; AMPK, 5′-AMP-activated protein kinase.
are decreased in patients with type II diabetes, which is always accompanied by elevated hepatic gluconeogenesis (21, 22), but it is unclear whether the decrease of plasma defensins contributes to the elevation of hepatic gluconeogenesis in diabetes. In this study, we tested the hypothesis that HNPs inhibit hepatic glucose production via a signaling pathway distinct from insulin.

**MATERIALS AND METHODS**

*Reagents—*HNP-1 and HNP-2 peptides with 1–6, 2–4, and 3–5 specific disulfide bonds were purchased from Sigma. Antibodies against Akt, phospho-Akt(Ser473), FoxO1, phospho-FoxO1(Ser256), phospho-c-Src(Y416), phospho-AMPK, AMPKα, or CSK were from Cell Signaling Technology, and antibodies against IRS1(Thr980) or β-actin were from Sigma. PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine; catalog number 529573) and PP3 (4-amino-7-phenylpyrazolo[3,4-d]pyrimidine; catalog number 529574) were from Calbiochem. The PEPPK promoter reporter construct was a kind gift from Dr. Jianhua Shao. The adenoviruses encoding a constitutively nuclear FoxO1 mutant (Ad-FoxO1-ADA) was a kind gift from Dr. Domenico Accili. The adenoviral vector encoding GFP was a kind gift from Dr. Robert J. Lefkowitz. All of the animal studies were approved by the Animal Care and Use Committee of the Hamner Institutes for Health Sciences and fully complied with the guidelines from the United States National Institutes of Health.

**Cell Cultures and Glucose Production via Gluconeogenesis in Hepatocytes—**Hepatoma cell lines including Hepa1c1c7, H411E, and HepG2 cells were cultured and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Primary hepatocytes from C57BL/6 mice were isolated as previously described (23–27). (Note that the mice were not fasted before hepatocyte isolations.) Hepatocytes in 24-well plates in William’s Medium E were washed with phosphate-buffered saline, and pretreated in serum-free medium at 75 min through the clamps. The blood samples (20 μl) were collected at the 0-, 60-, 90-, and 120-min time points of the clamps for the determination of plasma [3H]glucose, [3H2O, and 2-[14C]DG concentrations. Uptake of 2-[14C]DG by gastrocnemius muscle was measured as previously described (30).

**RNA Extraction and Real Time PCR—**Total RNAs from liver samples or cells were extracted by an RNase mini kit from Qia-gen and reverse-transcribed into cDNA. The target cDNAs were further quantified by TaqMan® real time PCR with specific probes from Applied Biosciences and normalized to levels of glyceraldehyde-3-phosphate dehydrogenase. The assay identification numbers for the probes and primers used in this study were Mm00440636_m1 (PEPCK), Mm00839363_m1 (G6Pase), Mm00447183_m1 (PGC-1α), and Mm99999915_g1 (glyceraldehyde-3-phosphate dehydrogenase).

**Immunoblotting and Measurements of Plasma Insulin—**As previously described (31–33), tissue or cell lysates were prepared with lysis buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 2 μg/ml leupeptin, and 10 μg/ml aprotinin, supplemented with 1 mM phenylmethylsulfonyl fluoride before use), resolved in

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All of the clamp studies were performed in male 11-week-old Zucker diabetic fatty rats (ZDF/Crl-Leprdb, strain code 370), purchased from Charles River Laboratories (Wilmington, MA). The ZDF rats were fed with a Purina 5008 diet. Indwelling catheters were placed into the right carotid artery and the left jugular vein with a standard procedure, and the animals were allowed to recover from the surgery for 3 days before clamp studies. All of the clamp studies were performed after a 16-h fast as previously described (28, 29). The synthesized HNP-1 peptide (2 μmol/kg of body weight) or same volume of saline solution (vehicle) was administered through the catheter in the left jugular vein 30 min prior to the clamp studies. During the 120-min clamps, hyperinsulinemia was achieved by a continuous infusion of human insulin (60 pmol/kg/min), whereas the blood glucose level was maintained at euglycemia via infusion of 20% glucose at various rates. The blood glucose levels were evaluated every 10 min. At the end of the clamps, an additional 100 μl of blood was collected for measurements of insulin and serum glutamic-oxaloacetic transaminase.

The levels of endogenous glucose production (EGP) during the clamp studies were measured as previously described (28, 29). Specifically, a prime-continuous infusion of [3-3H]glucose (8 μCi bolus, 0.11 μCi/min, catalog number TRK239; GE Healthcare) was performed during the clamps. The blood samples were collected at the 0-, 60-, 90-, and 120-min time points for measurements of blood glucose with a glucose meter (Ascensia Breeze 2; Bayer) and plasma [3H] by scintillation counting. To examine glucose uptake by skeletal muscle, a solution of 2-deoxy-2-[1-14C]glucose (2-[14C]DG; catalog number CFA-728; GE Healthcare) was administered as a bolus (10 μCi) at 75 min through the clamps. The blood samples (20 μl) were collected at the 80-, 85-, 90-, 100-, 110-, and 120-min time points of the clamps for the determination of plasma [3H]glucose, [3H2O, and 2-[14C]DG concentrations. Uptake of 2-[14C]DG by gastrocnemius muscle was measured as previously described (30).

**Treatment of Mice with HNP—**HNP-1 (0.4 mg/kg of body weight) dissolved in 100 μl of saline or the vehicle saline was administered to C57BL/6 mice that were fasted for 24 h via tail vein injection. Blood glucose levels were measured with tail blood. At the end of the experiments, all of the blood samples from sacrificed mice were collected for measurements of serum glutamic-oxaloacetic transaminase. Liver samples were collected and stored at −80 °C. Transcripts of PEPCK, G6Pase, and PGC-1α genes were measured by TaqMan® real time reverse transcription-PCR.
4–20% Tris-glycine gels, and transferred to nitrocellulose membranes (Bio-Rad). After blocking with 5% skim milk (GE Healthcare; catalog number 2501002), the membranes were incubated overnight with primary antibodies (1:1000 dilution). After extensive washes, the membranes were then incubated in 5% skim milk containing a 1:5000 dilution of the second antibody against rabbit IgG coupled to alkaline phosphatase (Sigma). Fluorescent bands were visualized with Typhoon 9410 variable mode Imager from GE Healthcare (Piscataway, NJ) and then quantified by densitometry using ImageQuant 5.2 software from Molecular Dynamics (Piscataway, NJ). Plasma levels of human and rat/mouse insulin were determined by enzyme-linked immunosorbent assay kits (Linco Research Inc., St. Charles, MO).

DNA Transfection, Luciferase Assay, and Adenoviral Infection—DNA plasmids were introduced into the indicated cells by Lipofectamine2000 transfection agents. Promoter activity was detected by a luciferase assay system (Promega) with a Wallac 1420 Multilabel Counter (PerkinElmer Life Sciences) and normalized to the protein level. Adenoviruses encoding the nuclear form of FoxO1 (Ad-FoxO1-ADA) or GFP (Ad-GFP), amplified in HEK-293 cells, were applied to infect primary hepatocytes at 10⁶ plaque-forming units/well in 6-well plates as described before (23).

Statistical Analysis—All of the data are presented as the means ± S.E. The data were evaluated for statistical significance by Student’s t test using GraphPad Prism version 5.0 for Windows (San Diego, CA). Differences at values of p < 0.05 were considered significant.

RESULTS

HNP-1 Reduces Blood Glucose Levels via Suppression of Hepatic Glucose Production in Both Normal Mice and ZDF Rats—To test the hypothesis that HNPs inhibit hepatic glucose production, normal mice were administered HNP-1 by tail vein injection for 0.5–8 h, followed by measurements of blood glucose and expression of key gluconeogenic genes in the liver. As shown in Fig. 1A, blood glucose levels were significantly reduced by HNP-1 in a time-dependent manner. Coincidently, hepatic levels of PEPCK and G6Pase transcripts were decreased (Fig. 1B). Levels of PGC-1α were not altered (Fig. 1B). These results suggest that HNPs can lower blood glucose levels in wild-type normal animals.

To determine whether or not HNPs can similarly diminish blood glucose levels in animals with insulin-resistant and diabetes, HNP-1 was administered to ZDF rats. As shown in Fig. 2A, blood glucose levels were significantly decreased by 1 h of treatment through infusion, indicating that HNPs can reduce blood glucose in diabetic animals with insulin resistance.

To examine the mechanism by which HNPs reduce blood glucose in diabetic animals, we examined the effect of HNP-1 on EGP and glucose uptake in skeletal muscle using the hyperinsulinemic-euglycemic clamp procedure. When blood insulin was clamped at a relatively stable level, HNP-1 increased the glucose infusion rate significantly (Fig. 2, B–F), suggesting either an increase in glucose uptake or a decrease of EGP or both. Disappearance of radiolabeled glucose from the blood and glucose uptake and utilization (glycolysis) were not altered significantly in skeletal muscle (gastrocnemius) (Fig. 2, G–I). Importantly, EGP was strongly suppressed by HNP-1 (Fig. 2J). It is noteworthy that HNP-1 at the concentrations used in this study did not cause detectable liver damage as measured by enzyme release (Fig. 2K). Together, these results support the notion that HNPs reduces blood glucose levels in insulin-resistant, diabetic animals primarily through inhibition of endogenous glucose production.

FIGURE 1. HNP-1 decreases fasting plasma glucose levels in normal mice. HNP-1 (0.4 mg/kg of body weight) or the vehicle solution was administered by tail vein injection to C57BL/6 mice that had been fasted for 24 h. Blood glucose levels (A) were measured, and transcripts of PEPCK, G6Pase, and PGC-1α genes in the liver (B) were quantified by TaqMan® real time reverse transcription-PCR.
HNPs Suppress Hepatic Glucose Production in Isolated Hepatocytes—To recapitulate the effect of HNPs on hepatic glucose production at a cellular level, isolated mouse hepatocytes were stimulated by cAMP/dexamethasone or forskolin in the presence or absence of HNP-1. Glucose production via gluconeogenesis was measured as detailed under “Materials and Methods” (24). As shown in Fig. 3 (A–C), HNP-1 significantly inhibited glucose production including gluconeogenesis and glycogenolysis although to a lesser extent than equimolar amounts of insulin. Similar results were observed with HNP-2 (data not shown).

HNP-1 Suppresses Transcription of Gluconeogenic Genes—Because hepatic gluconeogenesis is tightly controlled by the transcription of key genes encoding PEPCK and G6Pase (34), the effect of HNP-1 on expression of these genes was examined. As shown in Fig. 3D, HNP-1 significantly decreased transcripts for G6Pase and PEPCK genes induced by a cAMP-elevating agent, forskolin. We further examined the effect of HNP-1 on the activation of the PEPCK promoter. As shown in Fig. 3E, activation of the PEPCK promoter by forskolin was significantly suppressed by HNP-1 in a concentration-dependent manner. To control for the possibility that the inhibition of hepatic glucose by HNP-1 was caused by nonspecific cellular damage in the hepatocytes, the levels of LDH in the media were measured. As shown in Fig. 3F, HNP-1 at concentrations effective at suppressing gluconeogenesis and glycogenolysis did not evoke release of LDH from primary hepatocytes, whereas a higher concentration of HNP-1 (2 μM) caused a significant release of LDH. Together, these results show that HNP-1 can inhibit transcription of key gluconeogenic genes.

HNPs Stimulate Phosphorylation of Akt and FoxO1 in Hepatocytes—Because phosphorylation of Akt is a necessary event in the signaling cascade of insulin to suppress transcription of hepatic gluconeogenic genes (15), we measured levels of phosphorylated Akt in hepatoma cells (HepG2 and Hep1c1c7 cell lines) and primary mouse hepatocytes that had been treated with HNP-1, HNP-2, or insulin. As shown in Fig. 4 (A–C), both HNP-1 and -2 increased Akt phosphorylation, suggesting that HNPs suppress transcription of gluconeogenic genes through Akt.

Akt-mediated phosphorylation and the consequent exclusion of FoxO1 from the nucleus are necessary for insulin to inhibit hepatic gluconeogenesis (35, 36). Therefore, we examined levels of phosphorylated FoxO1 in the whole cell lysates of hepatocytes that had been treated with HNPs or insulin. Both HNP-1 and -2 stimulated phosphorylation of FoxO1 in Hepa1c1c7 cells and primary hepatocytes (Fig. 4, D and E). These results together support the notion that HNPs suppress gluconeogenesis through Akt and FoxO1.

To further examine the role of FoxO1 in HNP inhibition of gluconeogenesis, a mutant FoxO1 (Ad-FoxO1-ADA) that cannot be excluded from the nucleus (37, 38) was overexpressed in hepatocytes. The cells were then treated with forskolin in the presence or absence of HNP-1 or insulin. As shown in Fig. 4G, the presence of constitutively nuclear FoxO1 prevented the ability of both HNP-1 and insulin to suppress expression of G6Pase and PEPCK genes.

Because hepatic gluconeogenesis can also be inhibited by AMPK in an insulin-independent manner (39), we examined the possible involvement of AMPK. The levels of AMPK were measured in primary hepatocytes that had been treated with HNP-1, insulin, or metformin. As shown in Fig. 4H, HNP-1 did not activate AMPK, whereas the positive control metformin stimulated phosphorylation of AMPK.

HNPs Inhibit Hepatic Gluconeogenesis through a c-Src-dependent Signaling Pathway—To further examine the intracellular signaling pathway of HNPs in hepatocytes, we measured the levels of IRS1 tyrosine phosphorylation, which is essential for insulin-induced phosphorylation of Akt (reviewed in Ref. 40), and phosphorylation of c-Src, another known activator of Akt (41, 42). As shown in Fig. 5 (A and B), in contrast to insulin, which led to IRS1 tyrosine phosphorylation without significantly activating c-Src, HNP-1 and -2 failed to stimulate significant tyrosine phosphorylation of IRS1 but clearly stimulated phosphorylation of c-Src. These findings implicated a role for c-Src in HNP-1 suppression of hepatic gluconeogenesis. There-
fore, we used a chemical inhibitor of c-Src PP2 or the inactive analog PP3 prior to the treatment with HNP-1, followed by measurements of G6Pase gene transcripts. As shown in Fig. 5C, both HNP-1 and insulin significantly inhibited expression of the G6Pase gene induced by forskolin. Interestingly, blockade of c-Src activation prevented the HNP-1-induced inhibition of G6Pase gene expression without affecting insulin-induced suppression. We further examined the role of c-Src activation in the HNP-dependent inhibition of hepatic gluconeogenesis by a separate approach. CSK, a suppressor of c-Src kinase activation (43), was overexpressed in hepatocytes. As shown in Fig. 5D, CSK largely prevented the inhibitory effect of HNP-1 on glucose production via gluconeogenesis with no effect on insulin function. Together, these results indicate that HNP defensins inhibit hepatic gluconeogenesis through a c-Src-dependent signaling pathway.

DISCUSSION

As a major contributor to hyperglycemia in diabetes, hepatic glucose production is one of the primary targets for controlling blood glucose levels for the treatment of diabetes. Elevation of hepatic glucose production, particularly gluconeogenesis, in diabetes is primarily due to the loss of insulin function, which is generally considered to be mediated by an Akt-dependent signaling pathway (1). In this study, we found that HNPs can suppress hepatic glucose production via an Akt-dependent but insulin-independent signaling pathway and consequently reduce glucose production in both normal and diabetic animal models and isolated hepatocytes.

HNPs are released into the blood by granulocytes in response to bacterial infections (8). Normally, plasma levels of HNPs vary between 13.2 and 42 ng/ml (3.8–12 nM) (16, 17), which is well below the effective level (100–300 nM) of HNPs in suppression of hepatic gluconeogenesis. Therefore, under normal physiological conditions, it is unlikely that HNPs or other defensins play a significant role in the regulation of hepatic gluconeogenesis. However, under certain conditions such as severe bacterial infection (septicaemia and meningitis), plasma levels of HNPs in humans are dramatically increased and can reach as high as...
It has been observed that severe infections such as septicemia and bacterial meningitis are frequently accompanied by hypoglycemia (18–20). It is also known that severe bacterial infection can suppress expression of hepatic gluconeogenic genes without affecting plasma glucose levels (18, 44). The infection-induced inhibition of hepatic gluconeogenesis and the consequent hypoglycemia are currently believed to be related to tumor necrosis factor-α (19, 45). Nevertheless, our findings on the inhibitory role of HNP defensins in hepatic glucose production may provide a new or additional explanation for infection-induced hypoglycemia.

Interestingly, recent studies have shown that plasma levels of certain defensins are decreased in diabetic subjects, and the decrease has been suggested to be associated with the increased incidence of certain infec-

FIGURE 4. HNPs stimulate phosphorylation of Akt and FoxO1 in hepatocytes. A–F, hepatoma cells (HepG2 and Hepa1c1c7) and isolated mouse hepatocytes were treated with HNP-1 (100 nM or 300 nM), HNP-2 (100 nM or 300 nM), or insulin (10 nM) for 15 min as noted. Levels of phosphorylated Akt, FoxO1, and AMPK were then measured by immunoblotting with specific antibodies as indicated. Levels of total Akt, FoxO1, and AMPK in the same blots were also measured as loading controls. G, the constitutively nuclear form of FoxO1 (Ad-FoxO1-ADA) or GFP (control) were introduced into isolated hepatocytes 24 h before the cells were treated with forskolin for 2 h in the presence of HNP-1 or insulin as noted. Transcripts of G6Pase and PEPCK genes were quantified by TaqMan® real time PCR. All of the results represent three independent experiments.

FIGURE 5. HNPs suppress hepatic gluconeogenesis through c-Src tyrosine kinase. A and B, primary mouse hepatocytes were treated with HNP-1 (100 or 300 nM), HNP-2 (100 or 300 nM), or insulin (10 nM) for 15 min as noted. Levels of c-Src phosphorylation, IRS1 phosphorylation at tyrosine 896, total c-Src, and total IRS1 were measured by immunoblotting with specific antibodies as indicated. C, primary hepatocytes were pretreated with a c-Src kinase inhibitor, PP2, or an inactive analog, PP3, for 30 min prior to treatment with HNP-1 or insulin for 4 h. The cells were then stimulated with forskolin for another 2 h, followed by measurements of G6Pase gene transcripts by TaqMan® real time PCR. D, CSK, a suppressor of c-Src activation, was overexpressed in H4IIE hepatoma cells by transient transfection for 36 h. Glucose production via gluconeogenesis from these cells was quantified as detailed under “Materials and Methods” after the cells were treated with cAMP/dexamethasone (Dex) for 3 h in the presence or absence of a 30-min preincubation with either HNP-1 or insulin. The expression level of CSK was detected by immunoblotting with antisera against CSK. The results represent the means ± S.E. of two independent experiments, each in triplicate.
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tions in diabetes (21, 22). Although it is still unclear whether defensins play any role in the improvement of glycemic indices, it is intriguing that treatment of diabetic subjects with insulin can normalize plasma levels of defensins along with glycemic indices (22).

Insulin action is normally transmitted through a signaling cascade, which at least includes the insulin receptor, insulin receptor substrates such as IRS1/2, phosphatidylinositol 3-kinase, PDK1, Akt(PKB), and transcription factors such as FoxO1. Among these signaling components, IRS1 is a central substrate for many kinases such as c-Jun N-terminal kinase, p38, protein kinase Cs, and ribosomal S6 kinase that can serine phosphorylate IRS1 and desensitize insulin signaling (26, 46–48). Although the receptor for HNPs has not been identified, our results clearly show that HNPs can activate c-Src and consequently suppress hepatic glucose production in a c-Src-, Akt-, and FoxO1-dependent manner. The mechanism by which HNPs activate c-Src remains unknown. However, it appears that HNP activation of c-Src does not require IRS1. Thus, it is possible that utilization of HNPs or other similar reagents that activate c-Src might be used to bypass the blunted insulin signaling in diabetic patients with insulin resistance.

In conclusion, we have identified a suppressive role for HNPs in hepatic glucose production, and our results may provide an investigative avenue for finding new ways to overcome the blunted insulin signaling in patients with hepatic insulin resistance. Because the structures and functions of α defensins including HNPs are rather conserved among species (8), it is likely that the suppressive role of defensins is also conserved among different species. Identification of the HNP receptor and other HNP signaling components between c-Src and Akt in future studies may provide more valid targets for modulating glucose metabolism and treating diabetes.

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